Studies on Indirect Absorbance Detection of Anions by Capillary Electrophoresis

by

PHILIP DOBLE

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of

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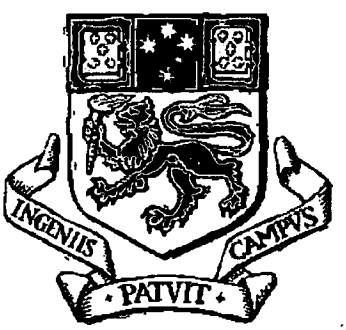

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LIST OF PUBLICATIONS

Type of Publication	Number	Reference
Review Articles	1	1
Papers in Refereed Journals	5	2-6
Posters at International Meetings	4	7-10
Oral Presentations at International Meetings	1	11

1 Doble, P.A., Haddad, P.R., Indirect photometric detection in Capillary Electrophoresis- A review, *Journal Of Chromatography A*, 1998 (in press).
(Chapter 1)

2 Doble, P.A., Haddad, P.R., Use of electrolytes containing multiple probe ions for the analysis of ions by capillary electrophoresis using indirect absorbance detection. *Analytical Chemistry,* 1998 (in press).
(Chapter 4)

3 Doble, P.A., Macka, M., Haddad, P.R., Factors influencing the choice of buffer in background electrolytes for indirect detection of fast anions by capillary electrophoresis, *Electrophoresis,* 1998 (in press).
(Chapter 6)

4 Doble, P.A., Macka, M., Haddad, P.R., Use of dyes as indirect detection probes for the high sensitivity determination of anions by capillary electrophoresis, *Journal Of Chromatography A,* 1998, *804*, 327.
(Chapter 5)

5 Doble, P.A., Macka, M., Haddad, P.R., Buffered chromate electrolytes for the analysis and indirect detection of inorganic anions in capillary electrophoresis, *Analytical Communications,* 1997, *34,* 351.
(Chapter 6)

6 Doble, P.A., Andersson, P., Haddad, P.R., (1997) Determination and prediction of transfer ratios for anions in capillary zone electrophoresis with indirect UV detection, *Journal Of Chromatography*, 1997, *770*, 291.
(Chapter 3)

7 Macka M., Doble P., Haddad P.R., Indirect Detection in CE: The Importance of Correctly Buffering Background Electrolytes, *Proc. International Ion Chromatography Symposium IICS '97, Santa Clara, CA, USA, 14.-17. September 1997, poster No. 105.*

8 Doble P., Macka M., Haddad P.R., Increased Sensitivity of Indirect Photometric Detection of Anions in CE using Highly Absorbing Dyes, *Proc. International Ion Chromatography Symposium IICS '97, Santa Clara, CA, USA, 14.-17. September 1997, poster No. 108.*

9 Doble P., Haddad P.R., Electrolytes Containing Multiple Probe Ions for the Analysis of Anions by Capillary Electrophoresis using Indirect UV Detection, *International Ion Chromatography Symposium IICS '97, Santa Clara, CA, USA, 14.-17. September 1997, poster No. 109.*

10 Doble P., Macka M., Andersson P., Haddad P.R., pH buffered electrolytes for indirect absorbance detection in CE: A study of separations of anions using a new buffered chromate electrolyte, *Proc. International Ion Chromatography Symposium IICS '96, Reading, England, 16.-19. September 1996, poster No. 115.*

11 Doble, P.A., Andersson, P., Haddad, P.R., Determination and prediction of transfer ratios for anions in capillary zone electrophoresis with indirect UV detection, *International Ion Chromatography Symposium IICS '96, Reading, England, 16.-19. September 1996.*

ABSTRACT

This work presents studies on separations and indirect absorbance detection of anions by capillary electrophoresis.

Transfer ratios (i.e. the number of moles of the UV-absorbing probe anion displaced by one mole of analyte anion) were determined for the separation of inorganic and organic anions by capillary zone electrophoresis using indirect UV absorbance detection. When the electrolyte was buffered and contained only the probe anion and a single counter-cation, transfer ratios calculated from Kohlrausch theory were found to agree well with values obtained experimentally from accurately determined mobility data.

Background electrolytes (BGEs) containing multiple co-anions were investigated as possible means to control peak symmetries and improve the sensitivity of indirect detection in the separation of a mixture of inorganic and organic anions having a range of electrophoretic mobilities. In general, it was found that an analyte mainly displaced the BGE component to which its mobility was closest, and exclusively displaced any BGE component having the same mobility. This behaviour was utilised to design BGEs containing multiple probes so as to improve peak shapes by matching the mobilities of the BGE components with those of the analytes. System peaks were observed for each multiple component BGE and for n BGE co-anions, n-1 system peaks were induced. A simple linear function relating the mobility of the system peak for a two co-anion BGE to the mobilities and relative concentrations of each of the co-anions was derived empirically.

High sensitivity for indirect detection was achieved by utilising highly absorbing species as the displaced co-ion. Two highly absorbing dyes, bromocresol green and indigo tetrasulfonate, were investigated as potential probes in the determination of small organic and inorganic anions. Minimal detectable amounts were in the low attomole region (1×10^{-18} mole), corresponding to sub μM vacuum injected solution concentrations. These detection limits were an order of magnitude lower than the general detection

limit reported for indirect photometric detection, and were comparable with detection limits achieved with indirect fluorescence detection.

Two approaches for buffering indirect electrolytes were investigated using chromate as a model system. The first system utilised a suitable buffering base as a counter-ion of the chromate. Two buffered electrolytes were investigated containing tris(hydroxymethyl)aminoethane (Tris, *pKa*=8.5) or diethanolamine (*pKa*=9.2). The analytical performance characteristics of the Tris buffered electrolyte were compared with the unbuffered chromate electrolyte. Both systems showed similar separation selectivity, efficiency and detection sensitivity, but the buffered electrolytes showed superior repeatability for migration times and peak areas, as well as a significantly greater tolerance to alkaline sample matrices.

The second buffering system investigated the suitability of the relatively slow (low absolute value of mobility) co-anionic buffer 2-(cyclohexylamino)-ethanesulfonic acid (CHES). Within its useful pH buffering range CHES acted as a competing probe co-anion. System peaks were induced which had deleterious effects on detection sensitivity of low to medium mobility anions. The mobility of the system peak was determined by the effective mobility of CHES, and increased with increasing pH. The peaks of analytes that migrated near or on the system peak were distorted and lost all quantitative properties. Analytes that migrated after the system peak either were not detected or exhibited a detector response which was reversed in direction. Analytes that migrated well before the system peak were unaffected.

LIST OF ABBREVIATIONS AND SYMBOLS

AU	absorbance units
BCG	bromocresol green
BGE	background electrolyte
Bis Tris	1,3-bis-[tris(hydroxymethyl)-methylamino]-propane
CAPS	3-(cyclohexylamino)propanesulfonic acid
CE	capillary electrophoresis
CHES	2-(cyclohexylamino)ethanesulfonic acid
CTAB	cetyltrimethylammonium bromide
CTAOH	cetyltrimethylammonium hydroxide
CZE	capillary zone electrophoresis
DEA	diethanolamine
DETA	diethylenetriamine
DMB	decamethonium bromide
DoTAOH	dodecyltrimethylammonium hydroxide
DTAB	dodecyltrimethylammonium bromide
emi	electromigration injection
EOF	electroosmotic flow .
HDMB	hexadimethrine bromide
HMB	hexamethonium bromide
HMOH	hexamethonium hydroxide
HPLC	High Performance Liquid Chromatography
HTAB	hexadecyltrimethylammonium bromide
IC	Ion Chromatography
ITS	potassium indigo-tetrasulfonate
LOD	limit of detection
MES	morpholinoethanesulfonic acid
m*M*	millimolar
MTAB	myristyltrimethylammonium bromide
NaOH	sodium hydroxide
NDSA	napthalene disulfonic acid
Nice Pak-OFM	Waters proprietry EOF modifer
OHB	hydroxy benzoic acid
OHBSA	hydroxybenzenesulfonic acid
S/N	signal to noise ratio
SDS	sodium dodecyl sulfate
SPA	sulfophthalic acid
TBAB	tetrabutylammonium bromide
TEA	triethanolamine
TEA	triethanolamine
THF	tetrahydrofuran
TMA	trimellitic acid
TR	transfer ratio
Tris	tris(hydroxymethyl)aminoethane
TSA	toluene sulfonic acid
TTAB	tetradecyltrimethylammonium bromide
TTAOH	tetradecyltrimethylammonium hydroxide
UV	ultra violet
Z1-methyl	Waters proprietry zwitter ion

μ	mobility
μm	micron

Table of Contents

1. Literature Review

1.1 Introduction and Scope of the Review

Indirect photometric detection in Capillary Electrophoresis (CE) is often employed for detection of cations and anions that lack suitable chromophores. In this form of detection, an absorbing co-ion called the probe (an ion with the same charge as the analyte), is added to the background electrolyte (BGE). Detection is accomplished by displacement of the co-ion leading to a quantifiable decrease in the background absorbance. The indirect detection method is universal in its applicability, and the instrumentation required is the same as for direct photometric detection, which is simple and commercially available.

Indirect absorbance detection was first introduced as a detection mode for CE by Hjerten *et al.* [1] in 1987. With the introduction of a sensitive universal detection scheme, the utility of CE has increased significantly. Indeed, the number of publications detailing applications of indirect photometric detection in CE has grown substantially in the past few years. Accordingly, the aim of this present literature review is to offer a critical summary of all publications dealing with the separation and indirect photometric detection (indirect absorbance and fluorescence) of anions by capillary electrophoresis that have appeared in abstracted journals up until the end of 1997. Discussions of indirect absorbance detection have been limited to factors that are relevant to this thesis. The scope of this review is defined as follows:

(i) Factors that influence the displacement process of ions for single and multiple co-ion electrolytes.

(ii) Buffering electrolytes for indirect absorbance detection of anions.

(iii) Factors influencing detection sensitivity.

(iv) Designing and optimising background electrolytes for indirect detection of anions.

(v) Separation of inorganic and small molecular weight anions using chromate as the probe.

(vi) Summary tables of all publications that have presented anions as analytes by indirect photometric detection regardless of the aim of their determination.

1.2 Kohlrausch's Regulating Function and the Transfer Ratio

The degree of displacement of the probe (co-ion) by the analyte is known as the transfer ratio (TR) [2]. The TR is defined as the number of moles of the probe displaced by one mole of analyte ions. As the detector response is proportional to the TR, a higher value of the TR results in a larger analyte peak area. On an intuitive level, one would expect displacement on an equivalent-per-equivalent basis so that, for example, the TR between a singly charged solute and a singly charged probe would be expected to be unity. Consequently, the peak areas for analytes of the same charge and concentration should be the same, allowing the theoretical possibility of calibration of each component without the need for separate calibration curves. However, Ackermans *et al.* [3] reported a non linear relationship between peak area and the effective mobilities of the ionic species for an equimolar sample composition. This behaviour can be explained by consideration of the electrophoretic separation mechanism for fully ionised ionic constituents, which can approximately be described by Kohlrausch's Regulating Function (ω) [4]:

$$\omega = \sum_i \frac{c_i.z_i}{\mu_i} = \text{constant} \qquad (1.1)$$

where c_i, z_i, and μ_i represent the ionic concentrations, absolute values of the charge, and absolute values of the effective mobilities of all ionic constituents, respectively.

Migration of ions through a capillary filled with a uniform electrolyte will be governed by one single ω function. This means that when an electric current is driven through the capillary the concentration profiles of all the ions remains the same as under the original conditions. If a sample is introduced with a single analyte the migration of ions is governed by two ω functions, the first associated with the sample plug, and the second with the bulk electrolyte. The ω function for each must

be constant, so that the concentration distributions of the ions for the bulk electrolyte and the sample plug remain as they were before the voltage was applied. That is, the flux of ions into the sample plug is exactly equal to the flux out.

A consequence of the ω functions is that the TR is dependent on the mobility of the probe, the analyte, and the counter ion. The relationship can be directly derived from the ω function [5-6] or consideration of the migration of ions using an eigenvalue approach [6-8].

Consider an electrolyte consisting of a single ion A, and its corresponding counter-ion, C, then from eqn. (1.1)

$$\omega_1 = \frac{c_A z_A}{\mu_A} + \frac{c_C z_C}{\mu_C}$$

where c_A and c_C are the concentrations of A and C in the background electrolyte.

For the electroneutrality condition: $c_A z_A = c_C z_C$

$$\therefore \qquad \omega_1 = c_A z_A \left(\frac{1}{\mu_A} + \frac{1}{\mu_C} \right) \quad = \quad \frac{c_A z_A}{\mu_A \mu_C} (\mu_A + \mu_C)$$

Now consider injection of an ionic analyte, BC, dissociated into a co-ion B, and counter-ion C. After an appropriate time the sample zone consists of A, B, and C. Then from equation (1.1):

$$\omega_2 = \frac{c'_A z_A}{\mu_A} + \frac{c_B z_B}{\mu_B} + \frac{c'_C z_C}{\mu_C}$$

where c'_A, c'_C are the concentrations of A, and C in the sample zone.

For the electroneutrality condition: $c'_A z_A + c_B z_B = c'_C z_C$

$$\therefore \qquad \omega_2 = \frac{c'_A z_A}{\mu_A} + \frac{c_B z_B}{\mu_B} + \frac{c'_A z_A}{\mu_C} + \frac{c_B z_B}{\mu_C}$$

$$= c'_A z_A\left(\frac{1}{\mu_A}+\frac{1}{\mu_C}\right)+c_B z_B\left(\frac{1}{\mu_B}+\frac{1}{\mu_C}\right)$$

Now $\omega_1 = \omega_2$

$$\therefore \quad c_A z_A\left(\frac{1}{\mu_A}+\frac{1}{\mu_C}\right) = c'_A z_A\left(\frac{1}{\mu_A}+\frac{1}{\mu_C}\right)+c_B z_B\left(\frac{1}{\mu_B}+\frac{1}{\mu_C}\right)$$

$$\left(c_A - c'_A\right) z_A\left(\frac{1}{\mu_A}+\frac{1}{\mu_C}\right) = c_B z_B\left(\frac{1}{\mu_B}+\frac{1}{\mu_C}\right)$$

Let $\Delta c_A = c_A - c'_A$

$$\therefore \quad \frac{\Delta c_A}{c_B} = \frac{z_B\left(\frac{1}{\mu_B}+\frac{1}{\mu_C}\right)}{z_A\left(\frac{1}{\mu_A}+\frac{1}{\mu_C}\right)}$$

$$= \frac{z_B}{z_A}\frac{\left(\mu_B+\mu_C\right)}{\left(\mu_A+\mu_C\right)}\cdot\frac{\mu_A\,\mu_C}{\mu_B\,\mu_C}$$

$$= \frac{z_B}{z_A}\cdot\frac{\mu_A}{\mu_B}\frac{\left(\mu_B+\mu_C\right)}{\left(\mu_A+\mu_C\right)} = \mathrm{TR} \qquad (1.2)$$

Several publications have attempted to validate the applicability of eqn. (1.2) in practical situations and to samples that contain more than one analyte, i.e. electrophoresis systems that consist of more than three components. Nielen [9] showed that indeed the response factors of alkylsulfate surfactants, analysed with veronal as the probe, fitted well with predictions made from eqn (1.2). Cousins *et al.* [2, 10] experimentally determined the TR values for a series of anions using a number of different probes. The experimental values of TR followed the general trend predicted by eqn. (1.2), but the fit was poor.

1.3 Peak Shapes

Peak shapes have been the subject of a number of papers [8, 11-14]. Mikkers *et al.* [5, 15] first described the effect of electrophoretic migration on analyte zone concentration distributions using a non-diffusional mathematical model derived from Kohlrausch's Regulating Function. The concentration distributions of the analyte bands were found to be dependent upon the relative mobility of the analyte and the BGE carrier co-ion. Analytes that have a higher mobility than the BGE co-ion migrate with a concentration distribution that is diffuse at the front and sharp at the rear of the zone, resulting in a fronting peak. The reverse holds true for analytes that are less mobile than the BGE co-ion, resulting in tailing peaks. Symmetrical peaks are obtained when the mobility of the co-ion and analyte are the same.

1.4 Multiple Co-ion Electrolytes

Currently, most BGEs designed for indirect absorbance detection consist of a single co-ion because the displacement process is relatively simple and well understood. In the previous discussions of the Kohlrausch Regulating Function, transfer ratios and peak shapes, BGEs containing only a single co-ion were considered. The question of what happens with the displacement process when the BGE contains two co-ions has been the subject of several publications. Wang and Hartwick [16] derived a theoretical model that indicated that when the mobility of an analyte was intermediate between the mobilities of two BGE co-ions, the analyte ion would mainly displace the co-ion to which its mobility was closest. When the analyte ion mobility was vastly different to either of the mobilities of the BGE co-ions competitive displacement occurred, i.e. no obvious discrimination of displacement took place. A consequence of this behaviour was that peak shapes for analytes of different mobilities could be improved by choosing two probe co-ions that matched the mobilities of the analytes. However, if one of the BGE co-ions was a UV transparent species, detection sensitivity was severely compromised for analytes that had a similar mobility to that of the transparent species. The authors also reported inducement of a system peak that had the potential to interfere with possible analytes of interest.

Beckers in a series of publications [13-14, 17] examined the system characteristics of BGEs that contain two co-ions for the analysis of cations. Initially, a two co-cation BGE was considered [17] that consisted of an UV transparent co-ion (potassium) and a single probe (histidine), with potassium having a higher mobility than histidine. The induced system peak always migrated between the mobilities of the two co-anions and its exact mobility was dependent upon the relative concentrations of each. UV transparent sample components that migrated before the system peak always migrated as a positive peak (i.e. increased absorbance) and analytes that migrated after the system peak migrated as a negative peak. Analytes that migrated near the system peak had distorted peak shapes and were enlarged, losing all quantitative properties. In a subsequent publication [13] the reverse situation was considered, i.e. a two co-cation BGE with a lower mobility UV transparent co-ion (Tris), and a fast probe (imidazole). Analytes that migrated before the system peak were negative (lowered absorbance) and peaks that migrated after the system peak were positive. The system peak behaved as it did in the former situation.

Macka *et al.* [18] developed some practical rules for predicting the existence of system peaks for the analysis of anions based on qualitative descriptions of transient isotachophoresis of the analyte species and of the co-ion to which its mobility was closest. Two cases were considered, the first being when the analyte had a higher mobility than either of the BGE co-ions and the second when the mobility of the analyte was slower than the co-ions. For both cases, it was demonstrated that the system peak was created by a vacancy of one component of the BGE that had the greatest difference in mobility relative to that of the analyte species. The authors also reported that a practical transition exists in which the BGE changes in behaviour from a single co-ion to a two co-ion BGE when the concentration of the second co-ion is approximately 5% of the concentration of the first (major) co-ion.

1.5 Buffering Indirect Electrolytes for the Analysis of Anions

Buffering electrolytes is essential for reproducible and rugged separations. One common method of buffering indirect electrolytes for the analysis of anions is achieved by utilising the probe itself as buffer. A weak acid is chosen as the probe

and the pH of the BGE is maintained near the *pKa* of the probe. Benzoate [19] and phthalate [20] are typical weak acid probes used for this method of buffering. The obvious disadvantages are: (i) the pH buffering range is limited to narrow regions approximately one pH unit either side of the *pKa* of the probe; (ii) the probe is partially ionised and therefore has low mobility and is useful only for the analysis of anions of intermediate to slow mobility and; (iii) the buffering capacity is limited due to the concentration of the probe.

Another method of buffering is the use of co-anionic buffers such as acetic acid [21], borate [20, 22-26], carbonate [27], and phosphate [28]. This approach would seem to overcome some of the disadvantages of the former method. However, the BGEs no longer contain a single co-anion, leading to potentially interfering system peaks and compromised sensitivity due to competitive displacement of the added co-anion (buffer). In studies where co-anionic buffers have been used, little emphasis has been placed on the appearance of system peaks and reduced sensitivity. The success of these separations have most likely been due to mobility selective displacement of the probe, because the authors have intuitively chosen probes with mobilities close to the analytes of interest, which provides the best peak shapes, maximises the transfer ratio, and minimises competitive displacement.

A further approach for buffering electrolytes for the analysis and indirect detection of anions is by use of a buffering counter-cation such as Tris [16, 29-37], and triethanolamine (TEA) [38-42]. These electrolytes are typically prepared by titration of the acid form of the probe with the buffering base to the *pKa* of the base. The advantage of this approach is that the BGE consists of a single co-anion (the probe), eliminating problems associated with multiple co-anion BGEs. However, the buffering capacity is still limited to the concentration of the probe.

Despite the desirability of buffering BGEs, a cursory review of the literature (see Tables 1.1 to 1.6) shows that most of the BGEs utilised are unbuffered. As capillary electrophoresis is a relatively new technique, most studies have been concerned with the feasibility of various separations, and as a consequence few methods have been validated by rigorous attention to reproducibility and ruggedness.

1.6 Optimising Limits of Detection

The limit of detection (LOD) for a non-absorbing analyte is given by [43]:

$$C_{lod} = \frac{C_p}{\mathrm{TR}\, D_r} = \frac{N_{BL}}{\mathrm{TR}\, \varepsilon\, l} \qquad (1.3)$$

where C_{lod} is the concentration limit of detection of the analyte, C_p is the concentration of the probe, TR is the transfer ratio (the number of moles of the probe displaced by one mole of the analyte), and D_r is the dynamic reserve (i.e., the ratio of the background absorbance to the noise), N_{BL} is the baseline noise, ε is the molar absorptivity of the probe, and l is the pathlength of the detection cell.

According to equation (1.3) C_{lod} can be optimised by reducing C_p or by increasing D_r. However for indirect absorption detection, D_r is also related to C_p so decreasing the probe concentration will not necessarily improve the detection limit, because D_r is simultaneously reduced [37]. Consequently, minimising C_{lod} often involves maximising D_r by either reducing the noise or increasing the BGE background absorbance.

Detection limits for indirect absorbance detection around 10^{-4} to 10^{-5} M are routinely achieved [44].

1.6.1 Noise

Conventional optical arrangements consist of UV lamps such as deuterium, cadmium and zinc etc. Light emitting diode based optical systems have been investigated as possible improvements in optical baseline noise over UV lamp sources [45]. These systems typically reduce noise levels by two to 10 times. A potential drawback is that these light sources emit in the visible region, which limits choice of BGE.

1.6.2 Pathlength

Increasing the pathlength, l, of the detection cell to increase the background absorbance has been the subject of several papers. Ma and Zhang [46] increased the pathlength by utilising capillaries of increasing diameter. They report that for a

narrow range of capillary diameters from 25 to 75 μm, the diameter is not a critical contribution to LODs. They speculate that the increase in joule heating with increasing capillary diameter increased the noise, negating any advantage obtained with the higher BGE background absorbance. This result was in disagreement with that reported by Steiner *et al.* [47]. They calculated signal to noise ratios (S/N) for capillaries from 10 to 10000 μm and showed that the S/N increased with capillary diameter. The discrepancy may be due to the fact that the contribution of joule heating to noise levels was neglected in the latter study, by measuring the background absorbance of the BGE in each capillary without the voltage on.

Weston *et al.* [48] extended the pathlength by use of a "bubble cell". A 75 μm capillary pathlength was extended to 300 μm by blowing a "bubble" at the detection end. For such an arrangement the influence of increased joule heating on noise is absent because the capillary diameter is unchanged over most of its length. They report that the detection sensitivity improved by approximately a factor of two, a result somewhat less than expected due to a concomitant increase in baseline noise.

1.6.3 Molar Absorptivity

Most publications report optimised detection limits by consideration of the molar absorptivity (ε) of the probe [2, 29, 41, 46, 48-54]. Increasing the molar absorptivity of the probe increases the dynamic reserve, and simultaneously reduces the necessary probe concentration, resulting in a lowering of detection limit. However, the probe must have a similar mobility to the analytes for the full benefit of high ε to be realised. Foret *et al.* [51] report a 50 times improvement in the detection limits of anions when the probe was changed from benzoate (low ε) to sorbate (high ε). Beck and Engelhardt [53] investigated a series of cationic probes for the analysis of inorganic and organic cations. Optimum conditions consisted of the probe with the closest mobility to the analytes and highest ε. Weston *et al.* [48] improved detection limits of inorganic cations by two to four times when changing the probe from UV Cat1 to UV Cat2. The improvement in detection was again due to the higher molar absorptivity of UV Cat2 and its closer mobility to the analytes.

Mala *et al* [52] utilised the highly absorbing dyes, chlorophenol red and methyl green for the analysis of inorganic cations, and indigo carmine for the analysis of inorganic anions. These dyes have molar absorptivities an order of magnitude higher than common probes such as phthalate and chromate, and were expected to decrease the detection limits by a similar degree. However, the reported detection limits were no better than those obtained with probes that are more conventional. The likely reason was due to presence of competing co-ions used as buffering agents.

1.7 Analysis of Inorganic and Small Molecular Weight Anions

Sodium chromate has been the most commonly used carrier electrolyte for the analysis of inorganic and small molecular anions. It has been applied to the analysis of anionic constituents in urine [55], Bayer liquors [56-58], Kraft black liquors [59-60], drinking water [61], brewed coffee [60], fine chemicals [60] and many other samples (see Table 1.1 p. 17).

Jones and Jandik [62] first used chromate for the determination of eight common inorganic anions: fluoride, carbonate, chloride, nitrite, bromide, nitrate, phosphate and sulfate. In a following study, [63] they investigated factors that controlled the selectivity of separation and extended the number of analytes separated (Figure *1.1*). The ionic strength of the BGE was found to have a limited influence on selectivity. Increasing the ionic strength increased the migration times of all the anions due to an accompanying decrease in EOF velocity. The migration order remained essentially the same with the exception of co-migration of sulfate and nitrite anion when the chromate concentration exceeded 7 m*M*. The pH of the BGE (pH 8 to 11) had little effect on the more strongly acidic anions (i.e. *pKa* values below 8). Weaker acids such as borate, carbonate and phosphate decreased in migration time with increasing pH due to a corresponding increase in ionisation. The concentration of the EOF modifier tetradecyltrimethylammonium bromide (TTAB) had pronounced effects on the relative migration times for bromide, sulfate and nitrate, but little influence for the rest of the anions. The authors speculate that ion pairing of these anions with the EOF modifier caused this effect.

Buchberger and Haddad [64] have reported that the migration order of inorganic anions was strongly influenced by the addition of organic solvents. A general increase in the migration time of all anions occurred due to a decrease in the electrical conductivity of the BGE, as well as slower electro-osmotic velocity because less of the EOF modifier was adsorbed onto the capillary wall. The resolution of the highly mobile ions thiosulfate, bromide and chloride decreased with increasing organic solvent concentration. The relative migration time of nitrite also increased with higher organic solvent concentrations, reversing the order of migration of nitrate and nitrite. The same authors [64] also investigated the effect of the alkyl chain length of the EOF modifier. Changes in the peak order were observed for the ions thiosulfate, iodide and thiocyanate when the alkyl chain length was sequentially increased from C12 to C16. The mechanism for this behaviour was unclear, although the authors speculated that the most probable cause was an ion interaction phenomenon between these anions and the EOF modifier. A further observation was that the average migration time of the anions decreased with increasing chain length of the EOF modifier.

Benz and Fritz [65] added 1-butanol to the BGE to aid in the reversal of the EOF. In previous studies [62, 63, 64] concentrations of the EOF modifier of 0.3 m*M* or more were found to be required to reverse the EOF. However, addition of 1-butanol up to 5% v/v reduced the required amount of modifier by a factor of 10. The authors report that separations using this approach exhibited less noise and greater reproducibility.

Harakuwe *et al.* [66] adjusted the selectivity of separation of inorganic anions by utilising binary surfactant mixtures, namely TTAB and dodecyltrimethylammonium bromide (DTAB). Adjusting the ratios of TTAB: DTAB was found to be a useful means to fine tune the separation. In a following study Haddad *et al.* [57] optimised the separation of inorganic and organic anions present in Bayer liquors. They reported that two optimal ratios of TTAB: DTAB existed in which most of the components of the Bayer liquor were separated, a result which was not achievable with the use of a single EOF modifier.

Although the separation selectivity has been studied extensively, most studies using the chromate electrolyte have involved the electrolyte being prepared from the sodium salt and therefore unbuffered. Surprisingly few publications have attempted to buffer the chromate electrolyte with the exception of several publications that have utilised the addition of a co-anionic buffer such as borate [22, 39, 67], and sodium carbonate [27]. In each of these studies, effects on detection sensitivity and inducement of system peaks by the addition of the co-anion were not considered.

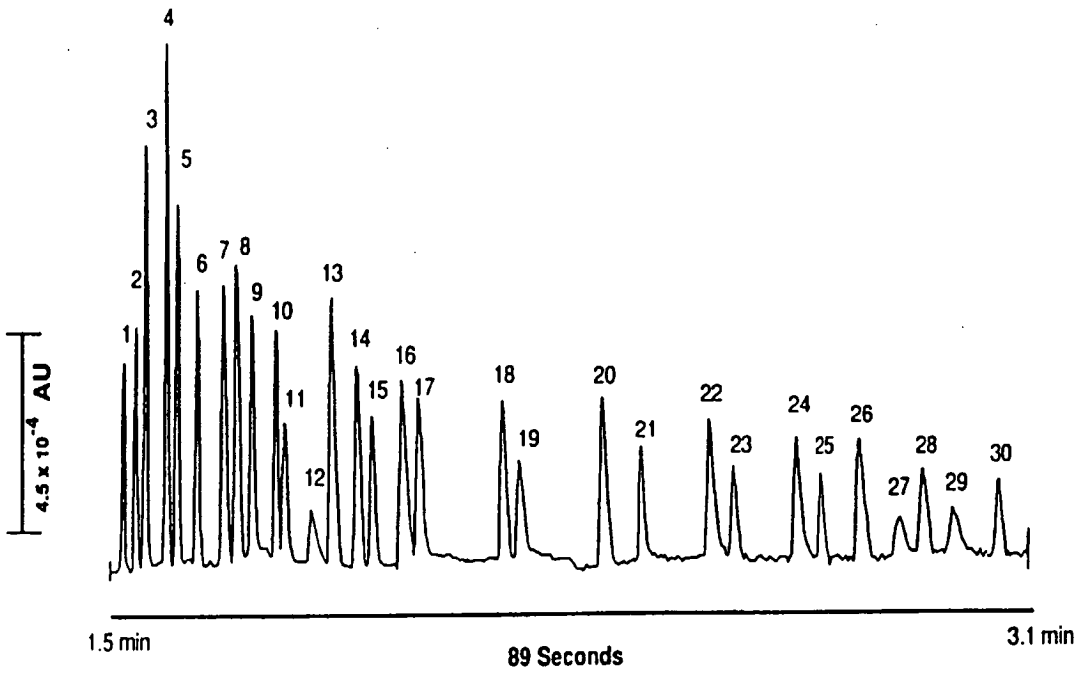


Figure 1.1 *Separation of anions using 5mM chromate, 0.5 mM TTAB , pH=8.0*
Key: 1= thiosulfate, 2= bromide, 3= chloride, 4= sulfate, 5= nitrite, 6= nitrate, 7= molybdate, 8= azide, 9= tungstate, 10= monofluorophosphate, 11= chlorate, 12= citrate, 13= fluoride, 14= formate, 15= phosphate, 16= phosphite, 17= chlorite, 18= galactarate, 19= carbonate, 20= acetate, 21= ethanesulfonate, 22= propionate, 23= propanesulfonate, 24= butyrate, 25= butanesulfonate, 26= valerate, 27= benzoate, 28= l-glutamate, 29= pentanesulfonate, 30= d-glucanate.
Reprinted from reference [63] with permission.

1.8 Conclusions

Some general conclusions can be made regarding the separation and indirect detection of anions by CE. The electrolyte conditions under which the theoretically derived values of the transfer ratio are in agreement with experimental values are not determined easily. Most separations of fast anions are achieved with BGEs that contain quaternary ammonium salts to reverse or suppress the electro-osmotic flow. The EOF modifiers are typically added up to 5% of the total concentration of the BGE. In effect, these electrolytes are multiple co-anion electrolytes due to the presence of the EOF modifier co-anion. The values of transfer ratio, and therefore possible effects on sensitivity, require more detailed investigation. Furthermore, the behaviour of separation systems that contain more than one co-anion is unpredictable. Improvements in peak shapes by exploiting mobility-selective displacement of probes for electrolytes containing multiple probes require further study.

Few studies have investigated the advantages of buffering electrolytes for method ruggedness and reproducibility of migration time and peak areas. Indeed, the majority of electrolytes are unbuffered. Although most studies report excellent reproducibilities, this is mainly achieved by frequent replenishment of the electrolyte reservoirs. Consideration of factors important for correct choice of buffering agent to avoid complications associated with the production of system peaks (for co-anionic buffers), and loss of sensitivity due to competitive displacement need to be investigated.

Finally, the sensitivity of indirect absorbance detection remains a weak point. However, this may be improved by employing highly absorbing dyes as probes to increase the dynamic reserve.

1.9 Aims of this project

The general objective of this work was to investigate separations and indirect detection of anions by capillary electrophoresis. Particular emphasis is placed on consideration of factors influencing correct design of buffered background

electrolytes without compromising detection sensitivity. Based on the above literature review, the detailed aims of this project were defined as follows:

(i) To investigate factors that influence the value of the transfer ratio, and in particular, elucidate electrolyte conditions under which maximum transfer ratios and therefore maximum sensitivities are obtained.

(ii) To investigate the effect on indirect detection and peak symmetry of BGEs containing multiple co-anions, and to gain a greater understanding of the competitive displacement process occurring in such BGEs.

(iii) To design buffered electrolytes containing highly absorbing probe species for sensitive determination of anions using indirect absorbance detection.

(iv) To investigate the suitability of buffering BGEs using counter-cation buffering bases in the analysis of inorganic and small molecular weight anions using chromate as a model electrolyte.

(v) To investigate the suitability of addition of slow co-anionic buffers for the analysis of inorganic and small molecular weight anions using chromate as a model electrolyte.

1.10 Overview of Indirect Photometric Detection

Tables 1.1 to 1.6 present a complete summary of all publications that have used indirect photometric detection. Probes, analytes, BGEs, detection wavelength, limits of detection (LOD), sample matrices and a brief description of the content of each citation are presented. The data is arranged according to alphabetical order of the probe and are grouped according to the scheme shown in Figure *1.2*. A separate entry is presented for each probe within each publication, so some references occur more than once. A listing of all abbreviations follows Table 1.6 (p. 51).

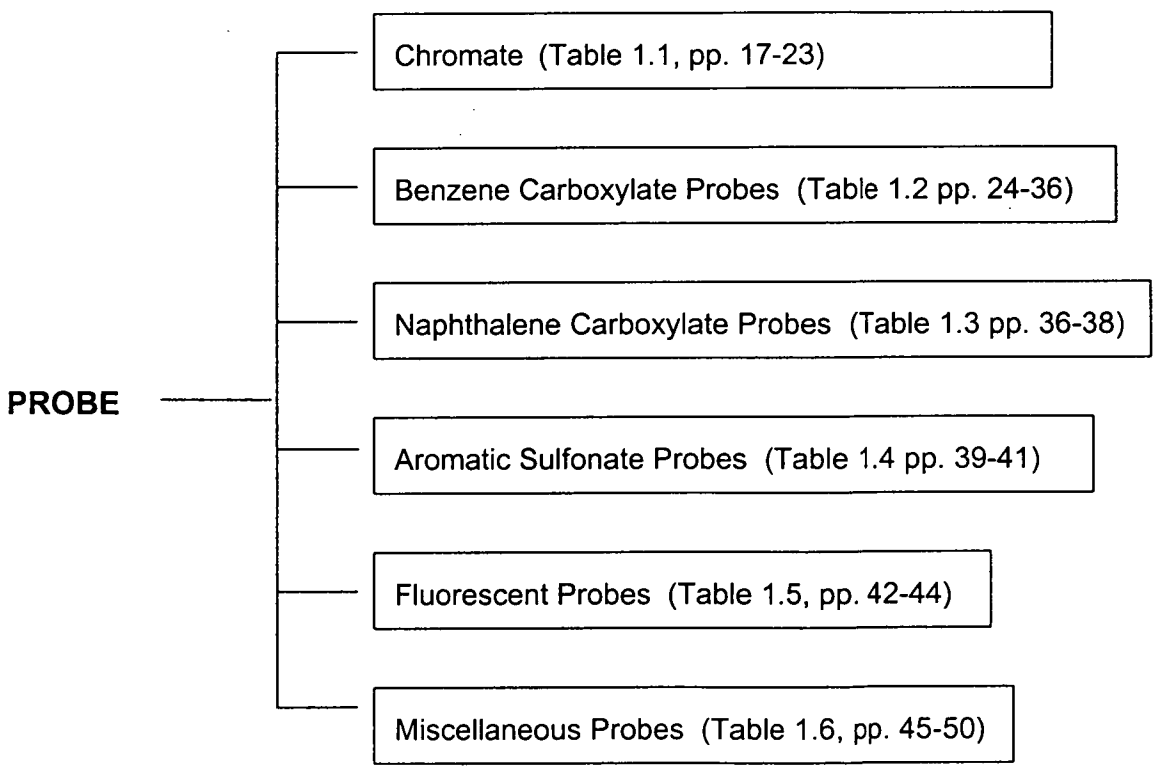


Figure 1.2 *Overview of indirect detection of anions schematic*

1.11 Probe and Analyte Data

The most crucial aspects for the success of separation and indirect detection of any analyte are: (i) mobility of probe, (ii) mobility range of the analytes, (iii) and sensitivity of detection. For optimum peak shapes, the mobility of the probe must be as close as possible to the mobility of the analytes. Sensitivity of detection is greatest when the molar absorptivity of the probe is high. Accordingly, Table 1.7 (pp. 52-56) is a complete listing of probes and Table 1.8 (pp. 57-66) a complete listing of analytes in all publications in abstracted journals that utilised indirect photometric detection. Where possible, the molar absorptivity (for probes), *pKa*, and mobility of each probe or analyte, and the pH at which the mobility was measured are listed. The cross-references detail publications where the probe or analyte has been cited in any publication other than from where the data were taken. Both tables are sorted alphabetically by the probe or analyte.

Table 1.1 Chromate

Analytes	BGE	Detection Wavelength (nm)	LOD	Matrix	Comments	Ref.
acetate, ascorbate, citrate, glucanate, glutamate, lactate, malate, oxalate, tartrate	5 m*M* chromate, 0.5 m*M* TTAB, pH=8.0	220, 240, 265	n/a	beverages	investigation of suitable probes for the analysis of organic acids	[68]
sulfate, sulfide, tetrathionate, thiosulfate	3 m*M* Chromate, 10 m*M* Tris, 0.5 m*M* DETA, pH=8.15	254, 214	2-4 μ*M*	natural water	optimisation of BGE for the analysis of sulfur species	[29]
C2-C14 fatty acids	5 m*M* chromate, Tris, 30%THF, pH=8.2	254, 270, 300	1 -2.5 μ*M*	cocoa oil extract	comparison of probes for the analysis of C2-C14 frees fatty acids; method validation for anisate as probe for the analysis of cocoa oil extract	[31]
acetate, bromide, butyrate, chloride, formate, nitrate, nitrite, oxalate, propionate, sulfate	5.0 m*M* chromate, 0.5 m*M* TTAB pH=8	254, 280	100-200 ng/ml	atmospheric aerosols	comparison of probes for the analysis of organic and inorganic anions; comparison of CE and IC	[38]
bromide, chloride, citrate, fluoride, phosphate, sulfate	5 m*M* chromate, 0.5 m*M* TTAB, pH=8.0	254	n/a	standard solutions	evaluation of BGE for low molecular mass anions	[2]
Inorganic anions, alkyl sulfates, organic acids	5.0 m*M* chromate, 0.5 m*M* TTAB pH=8.0	254	n/a	Kraft black liquor, dental plaque extract, human saliva, butyric acid extract of air filter,	optimisation of analysis for anions in real samples	[69]
arsenate, arsenite, ascorbate, bromide, carbonate, chloride, citrate, fluoride, nitrate, nitrite, oxalate, phosphate, sulfate	5 m*M* chromate, 0.5 m*M* TTAB, pH=8,10	254	n/a	urine	method for the analysis of anion constituents in urine	[55]

See p. 15 for notes on organisation of this Table. See p. 51 for abbreviations. See p. 67 for References.

Table 1.1 (continued) Chromate

Analytes	BGE	Detection Wavelength (nm)	LOD	Matrix	Comments	Ref.
acetate, chloride, fluoride, formate, nitrate, oxalate, phosphate, propionate, sulfate	10 m*M* chromate, 0.5 m*M* pH=8.0	254	0.3 - 0.8 ppb emi	nuclear power plant water	method for the determination of trace amounts of anions with electromigration preconcentration	[70]
acetate, carbonate, carbonate, chlorate, citrate, fluoride, formate, malonate, oxalate, phosphate, succinate, sulfate, tartrate	5.5-7.5 m*M* chromate, 3-5 m*M* TTAB, 1-3 m*M* DTAB, pH=9.1	254	0.1 - 0.4 µg/ml	Bayer liquor	optimisation of separation of components of Bayer liquors by consideration of mixtures of EOF modifiers	[57]
bromide, chloride, fluoride, nitrate, nitrite, phosphate, phosphite, sulfate	4.5 m*M* chromate, 0.4 m*M* TTAB	254	n/a	drinking water	comparison of IC method and CIA method for drinking water	[71]
chloride, fluoride, nitrate, oxalate, phosphate, sulfate	10 m*M* chromate, 1.5 m*M* TTAOH pH=11.0 7.0 m*M* chromate, 0.5 m*M* TTAOH, pH=8.0	254	0.4 - 1.2 µg/l emi	hard disk drive heads	method for the low level determination of anions using electromigration injection	[72]
bromide, chlorate, chloride, fluoride, iodide, nitrate, nitrite, sulfate, thiocyanate, thiosulfate	various	254	n/a	standard solutions	investigation of carrier electrolyte on separation selectivity of inorganic anions	[64]
oxalate, sulfate, chloride	5 m*M* chromate, 0.5 m*M* TTAB, 1 *M* Z1-methyl, pH=10.5	254	n/a	Bayer Liquor, vegetation forage sample	methods for the analysis of ions important to the aluminium industry by both CE and IC	[56]
bromide, chloride, nitrate, nitrite, sulfate	5 m*M* chromate, 0.5 m*M* TTAB	254, 214	n/a	drug counter ions	comparison of IC and CE for the analysis of drug counter ions	[73]
inorganic anions, organic acids, carbohydrates, alkyl sulfonates	5 m*M* chromate, 0.4 m*M* TTAB, pH=8.0, 11	254	n/a	coffee, Kraft Black liquor	analysis of anions with difficult sample matrices including coffee, and Kraft Back Liquor	[60]

See p. 15 for notes on organisation of this Table. See p. 67 for References. See p. 51 for abbreviations.

Table 1.1 (continued) Chromate

Analytes	BGE	Detection Wavelength (nm)	LOD	Matrix	Comments	Ref.
chloride	5 m*M* sodium chromate, 0.5% w/v hydroxypropyl(methylcellulose), pH=7.0 with sulfuric acid	273, 214	n/a	rat airway surface	method for the analysis of cations and anions of rat airway surface with direct sample injection	[74]
ethyl methylphosphonic acid, isopropyl methylphosphonic acid, methylphosphonic acid, pinacolyl methylphosphonic acid	4.5 m*M* chromate, 1.0 m*M* sodium hydrogen carbonate, 0.5 m*M* TTAB, pH=9.2	254	n/a	groundwater	method for the analysis of nerve agent degradation products in groundwater	[27]
citrate, diethylenetriaminepentacetic acid, ethylenediaminetetraacetic acid, nitrilotriacetic acid, phosphate, pyrophosphate, tripolyphosphate	5.0 m*M* potassium chromate, 0.05 m*M* CTAB, pH=3.5	260	20-50 μ*M*	commercial detergent	separations of polyphosphates and polycarboxyclic acids	[75]
acetate, butyrate, carbonate, chloride, formate, oxalate, propionate, sulfate, sulfite, thiosulfate	5 m*M* chromate	254, 214	n/a	Kraft black liquor	method for the analysis of anions and cations in Kraft black liquor for the pulp and paper industry	[59]
bromide, chloride, fluoride, nitrate, nitrite, phosphate, sulfate	5 m*M* sodium chromate, 0.5 m*M* CTAB, pH=7.9	254	n/a	caustic solutions, Hydrochloric acid digests	methods for sample cleanup using membrane based solid phase extraction before CE analysis	[76]
bromide, chlorate, chloride, fluoride, nitrate, sulfate	7.5 m*M* potassium chromate, 0.2 m*M* TTAOH, pH=8.5	211, 254	n/a	silicon wafer surfaces	method for the determination of inorganic anions on wafer surfaces after dissolution with hydrofluoric acid vapour	[77]
organic acids, inorganic anions, alkyl sulfonic acids	5 m*M* chromate, 0.5 m*M* TTAB, pH=8.0	254	n/a	standard solutions	considerations of selectivity changes in the separation of ions	[63]

See p. 15 for notes on organisation of this Table. See p. 67 for References. See p. 51 for abbreviations.

Table 1.1 (continued) Chromate

Analytes	BGE	Detection Wavelength (nm)	LOD	Matrix	Comments	Ref.
chloride	5 m*M* chromate, 3 m*M* borate, pH=9.2	254	n/a	standard solutions	separation of chloride isotopes	[67]
arsenate, arsenite, dimethylarsinate, monomethylarsonate	6 m*M* chromate, 1:40 v/v nice pak OFM, pH=8.0	254	3.5-15 pg	coal fly ash	analysis of arsenic species in coal fly ash samples	[78]
acetate, borate, bromide, butyrate, carbonate, chloride, citrate, fluoride, formate, molybdate, nitrate, nitrite, oleate, phosphate, propionate, sulfate, thiosulfate, tungstate	10 m*M* chromate, 2.30 m*M* CTAB, pH=11.5	254	0.32 µg/ml	vegetables	validation of method for the analysis of nitrate and nitrite in vegetables	[79]
bromide, carbonate, chloride, fluoride, iodate, iodide, nitrate, nitrite, phosphite, sulfate	10 m*M* chromate, 2.3 m*M* CTAB, pH=11.5	254	n/a	standard solutions	prediction of migration behaviour of anions when in the presence of micelles; the micelles are formed when the EOF modifier is above its CMC	[80]
arsenate, arsenite	5 m*M* chromate, 0.25 m*M* HTAB to pH 10 with Tris	254, 214	25-50 µ*M*	standard solutions	comparison of indirect detection and X -ray detection	[34]
acetate, carbonate, chlorate, chloride, formate, nitrate, sulfate	5 m*M* potassium chromate, 0.2 m*M* TTAB	276,220	3-450 ng/ml	rain drops	comparison of chromatographic methods for the analysis of cations and anions in rain drops	[81]
sulfate, fluoride, nitrite, nitrate, chloride, bromide	5 m*M* sodium chromate, 0.004-0.6 % of three cationic polyelectrolytes	254	< 10 µ*M*	standard solutions	selectivity effects with addition of cationic polyelectrolytes to the BGE	[82]
bromide, chloride, nitrate, nitrite, phosphate, sulfate	5 m*M* sodium chromate, 0.4 m*M* HMB, or TTAB, or CTAB, or HDMB, pH=8.3	254	200 - 1000 µg/l	standard solutions	study on effects of 4 different EOF modifiers	[83]
bromate, carbonate, chloride, fluoride, iodide, nitrate, nitrite, phosphate, sulfate, thiocyanate	5 m*M* chromate, 2.5 m*M* TTAB, pH=8 to 13	254	n/a	standard solutions	effect of pH on selectivity of selected inorganic anions	[84]

See p. 15 for notes on organisation of this Table. See p. 67 for References. See p. 51 for abbreviations.

Table 1.1 (continued) Chromate

Analytes	BGE	Detection Wavelength (nm)	LOD	Matrix	Comments	Ref.
acetate, azide, bromide, butyrate, carbonate, chlorate, chloride, chlorite, fluoride, formate, molybdate, nitrate, nitrite, propionate, sulfate, valerate	5 m*M* chromate, .075 m*M* TTAB, 5 % butanol, pH=8	254	n/a	standard solutions	discussion of effects of butanol as EOF modifier	[65]
arsenate, arsenite, chloride, fluoride, selenate, selenite, sulfate	5.0 m*M* chromate, 0.25 m*M* HTAB, pH=10.0 with Tris	254	n/a	standard solutions	method for speciation of heavy metals	[35]
acetate, chloride, citrate, fumarate, nitrate, phosphate, sulfate	5.0 m*M* chromate, 0.4 m*M* TTAB, pH=8	254, 185	150-320 ng/ml	vitamin tablet.	method validation for the analysis of anions and cations in vitamin formulations; comparison of CE and ICP	[85]
bromide, carbonate, chlorate, chloride, fluoride, nitrate, nitrite, phosphate, sulfate	3 m*M* potassium dichromate, 1.6 m*M* TEA, 0.4 m*M* TTAB or DMB or TBAB pH=8.0	214, 260	n/a	standard solutions	optimisation of separation of inorganic cations and anions by capillary electrophoresis (French)	[86]
bromide, chlorate, chloride, fluoride, nitrate, nitrite, phosphate, sulfate	sodium chromate with various modifiers, buffers etc.	260	n/a	mineral waters	review and experimental on adjusting the selectivity of inorganic anions using chromate as the probe	[87]
acetate, bromide, carbonate, chloride, fluoride, molybdate, nitrate, phosphate, sulfate, thiosulfate	2-12 m*M* sodium chromate, 3.5-10 m*M* CTAB, pH=6.5-12	254	0.12 - 0.84 ppm	water	optimisation of pH, EOF modifier and probe concentration for the analysis of anions with chromate as probe	[88]
carbonate, chloride, nitrate, sulfate	7 m*M* chromate, 0.5 m*M* TTAB, pH=8.1	254	< 5 µg/ml	dye solution, power plant water	comparison of anion analysis by CE and IC	[89]
oxalate	5 m*M* chromate, 0.5 m*M* TTAB, pH=8.0	254	n/a	Bayer Liquor	evaluation of method for the analysis of oxalate in Bayer Liquors	[58]

See p. 15 for notes on organisation of this Table. See p. 67 for References. See p. 51 for abbreviations.

Table 1.1 (continued) Chromate

Analytes	BGE	Detection Wavelength (nm)	LOD	Matrix	Comments	Ref.
iodate, bromide, carbonate, chloride, fluoride, iodide, nitrite, phosphate	10 m*M* sodium chromate, 2.3 m*M* CTAB		n/a	vegetable sample, pine needles	discussion of method development for small ions	[90]
chloride, morpholine, nitrate, N-morpholine, N-morpholine-N-oxide, sulfate	10 m*M* chromate, pH=7.0 5 m*M* chromate, pH=8.0	255	5-70 ppm	standard solutions	method for the analysis of inorganic anions and morpholine derivatives	[91]
adamantane carboxylic acid, adamantane dicarboxylic acid, chloride, nitrate	5.0 m*M* sodium chromate, 0.2 m*M* CTAB, pH=11.3	254	n/a	standard solutions	method for the determination of binding constants for adamantane carboxylic acids and beta cyclodextrin derivatives	[92]
bromide, carbonate, chloride, fluoride, nitrate, nitrite, phosphate, sulfate	5 m*M* chromate, DTAB or TTAB, pH8.8	254	n/a	Bayer liquor	selectivity changes due to EOF modifier	[66]
ethoxylated alcohol sulfates	1m*M* potassium chromate, 1 m*M* sodium tetraborate, 30 m*M* boric acid 1 m*M* potassium dichromate, 1 m*M* sodium tetraborate, to pH=8 with boric acid, 30 % v/v acetonitrile.	265	n/a	commercial dishwashing formulation	method for the analysis of ethoxylated alcohol sulfates	[22]
bromide, chloride, fluoride, nitrate, nitrite, phosphate, sulfate	4 m*M* chromate, 0.3 m*M* TTAB, pH=8.1	254	n/a	tapwater, wellwater, industrial wastewate, power plant waste water	method for the analysis of inorganic anions in water; results between IC and CE are compared	[61]
acetate, bromide, chlorate, chloride, fluoride, molybdate, nitrate, nitrite, sulfate	5 m*M* chromate	254	n/a	standard solutions	comparison of polypropylene and fused silica capillaries for the analysis of inorganic anions	[93]

See p. 15 for notes on organisation of this Table. See p. 67 for References. See p. 51 for abbreviations.

Table 1.1 (continued) Chromate

Analytes	BGE	Detection Wavelength (nm)	LOD	Matrix	Comments	Ref.
chloride, fluoride, monofluorophosphate, nitrate, phosphate, sulfate, tungstate	10 m*M* sodium chromate, 0.1 m*M* CTAB, pH=9.37	254	0.1-0.4 µg/ml	toothpaste	comparison of analysis of fluoride and monofluorophosphate in toothpaste by CE and IC	[94]
bromide, chloride, nitrate, nitrite, sulfate, thiosulfate	4 m*M* sodium chromate	254, 226, 270	n/a	standard solutions	evaluation of coated capillaries for the analysis of fast anions without the addition of EOF modifiers	[95]
bromide, butanesulfonate, carbonate, chloride, citrate, ethanesulfonate, hexanesulfonate, molybdate, nitrate, nitrite, pentanesulfonate, phthalate, propanesulfonate, sulfate, tungstate	5 m*M* chromate, 0.5 m*M* TTAB, pH=8.0	254	n/a	standard solutions	method development strategies for analysis of anions	[96]
bisinositol phosphate, hexakisinositol phosphate, monoinositol phosphate, trisinositol phosphate	2.5 m*M* potassium chromate, 0.5 m*M* TTAB, 5 m*M* borate, pH=7.3	270, 254	200 ng/ml	physiological samples	investigation of feasibility to analise for inositol phosphates by CE	[20]
bromide, chloride, fluoride, nitrate, nitrite, phosphate, sulfate, thiosulfate	5 m*M* sodium chromate, 5 m*M* boric acid, 0.5 m*M* TTAB, pH=8.0	254	1-3 mg/l	standard solutions	validation of CE method for the analysis of anions	[39]

See p. 15 for notes on organisation of this Table. See p. 67 for References. See p. 51 for abbreviations.

Table 1.2 Benzene Carboxylate Probes

Probe	Analytes	BGE	Detection Wavelength (nm)	LOD	Matrix	Comments	Ref.
p-aminobenzoate	alanine, asparagine, aspartic acid, cysteine, glutamic acid, glutamine, glycine, histidine, iso leucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, valine	5 -10 m*M* p-aminobenzoate, pH=10-11.2	222-288	n/a	standard solutions	investigation of nine probes for the analysis of amino acids	[98]
p-aminobenzoate	acetate, chloride, nitrate, oxalate, succinate	7.5 m*M* p-aminobenzoate, 750 μ*M* barium hydroxide, 100 μ*M* TTAOH, pH=9.4	264, 220	n/a	rain drops	analysis of anions and cations in single raindrops	[99]
p-aminosalicylate	alanine, asparagine, aspartic acid, cysteine, glutamic acid, glutamine, glycine, histidine, iso leucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, valine	5 - 10 m*M* sorbate, pH=10-11.2	222-288	n/a	standard solutions	investigation of nine probes for the analysis of amino acids	[98]
Anisate	C2-C14 fatty acids	10 m*M* anisate, 20 m*M* Tris, 0.75 m*M* trimethyl-beta-cyclodextrin, pH=8.2	254, 270, 300	1-2.5 μ*M*	cocoa oil extract	comparison of probes for the analysis of C2-C14 frees fatty acids; method validation for anisate as probe for the analysis of cocoa oil extract	[31]
Benzoate	alanine, asparagine, aspartic acid, cysteine, glutamic acid, glutamine, glycine, histidine, iso leucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, valine	5 - 10 m*M* benzoate, pH=10-11.2.	222-288	n/a	standard solutions	investigation of nine probes for the analysis of amino acids	[98]

See p. 15 for notes on organisation of this Table. See p. 67 for References. See p. 51 for abbreviations.

Table 1.2 (continued) Benzene Carboxylate Probes

Probe	Analytes	BGE	Detection Wavelength (nm)	LOD	Matrix	Comments	Ref.
Benzoate	alpha cyclodextrin, beta cyclodextrin, gamma cyclodextrin	100 m*M* benzoic acid, 100m*M* Tris, pH=7	254	50 μ*M*	fermentation broth, urine, plasma, pharmaceutical preparations	method for the analysis of cyclodextrins	[30]
Benzoate	bromide, chloride, nitrate, sulfate	0.02 *M* benzoate, pH=6.5	228	0.3-1.1 fmol	standard solutions	strategies to optimise indirect UV detection of anions	[46]
Benzoate	C2-C14 fatty acids	40 m*M* benzoate, Tris, 30%THF, pH=8.2	254, 270, 300	1-2.5 μ*M*	cocoa oil extract	comparison of probes for the analysis of C2-C14 frees fatty acids method validation for anisate as probe for the analysis of cocoa oil extract	[31]
Benzoate	sulfoalkyl ether beta-cyclodextrin derivatives	30 m*M* benzoate, pH 6.0 with Tris	254	n/a	standard solutions	method which characterizes the degrees of substitution of heterogeneous sulfoalkyl ether beta-cyclodextrin derivatives	[100]
Benzoate	(N-morpholino)ethane-sulfonic acid, acetate, butanoate, chlorate, chloride, fluoride, formate, hexanoate, pentanoate, propionate	0.01 *M* benzoic acid adjusted to pH 8 with Tris	254	n/a	standard solutions	theoretical considerations of relationship between effective mobilities and peak area for indirect detection	[3]
Benzoate	acetate, butyrate, formate, hexanoate	10 m*M* imidazole to pH 7 with benzoic acid	214	n/a	standard solutions	theoretical considerations for the calculation of sample zones; constructing simulated electropherograms	[101]
Benzoate	alpha cyclodextrin, beta cyclodextrin, gamma cyclodextrin	30 m*M* benzoic acid, Tris, pH=6.2	254	n/a	Cicladol tablet	method for the analysis of cyclodextrins	[32]

See p. 15 for notes on organisation of this Table. See p. 67 for References. See p. 51 for abbreviations.

Table 1.2 (continued) Benzene Carboxylate Probes

Probe	Analytes	BGE	Detection Wavelength (nm)	LOD	Matrix	Comments	Ref.
Benzoate	aspartate, butyrate, chlorate, chloride, dichloroacetate, dimethylmalonate, glucuronate, glutamate, hydroxyisobutyrate, lactate, malonate, methylmalonate, phosphate	0.02 *M* benzoic acid, histidine, pH=6.2, 0.1 % triton X 100	254	n/a	standard solutions	theoretical considerations of indirect detection, with some practical examples	[51]
Benzoate	inorganic anions, alkyl sulfates, organic acids	10 m*M* benzoate, 0.5 m*M* TTAB, pH=6.0	254	n/a	Kraft black liquor, dental plaque extract, human saliva, butyric acid extract of air filter	optimisation of analysis for anions in real samples	[69]
Benzoate	4-sulfobutyl ether derivatives of beta cyclodextrin	30 m*M* benzoic acid-Tris pH=6.0	230	n/a	reaction mixtures	method for characterisation of ether derivatives of beta cyclodextrin	[102]
Benzoate	alitame, aspartame, cyclamate	10 m*M* benzoate, 1 m*M* CTAOH, pH=6.6	254	n/a	cordial, softdrink, jam	method for the analysis of aspartame in food products	[19]
Benzoate	bromide, chloride, citrate, fluoride, phosphate, sulfate	5 m*M* benzoate, 0.5 m*M* TTAB, pH=8.0	254	n/a	standard solutions	evaluation of BGE for low molecular mass anion	[2]
Benzoate	acetate, ascorbate, citrate, glucanate, glutamate, lactate, malate, oxalate, tartrate	5 m*M* benzoate, 0.3 m*M* TTAB, pH=8.0	220, 240, 265	n/a	beverages	Investigation of suitable probes for the analysis of organic acids	[68]
o-benzylbenzoic acid	bromide, chloride, nitrate, sulfate	0.02 *M* o-benzylbenzoic acid, pH=6.5	228	0.3-1.1 fmol	standard solutions	strategies to optimise indirect UV detection of anions	[46]

See p. 15 for notes on organisation of this Table. See p. 67 for References. See p. 51 for abbreviations.

Table 1.2 (continued) Benzene Carboxylate Probes

Probe	Analytes	BGE	Detection Wavelength (nm)	LOD	Matrix	Comments	Ref.
2,3-dihydroxybenzoic acid	C2-C14 fatty acids	10 m*M* 2,3-dihydroxybenzoate, Tris, 30%THF, pH=8.2	254, 270, 300	1-2.5 μ*M*	cocoa oil extract	comparison of probes for the analysis of C2-C14 frees fatty acids; method validation for anisate as probe for the analysis of cocoa oil extract	[31]
2,4-dihydroxybenzoic acid	dodecyl sulfate	5 m*M* 2,5-dihydroxybenzoic acid, 5 % methanol, pH=8.1 with NaOH	250	<0.8 mg/l	stream water	method for the determination of sodium dodecyl sulfate in stream water	[103]
2,5-dihydroxybenzoic acid	chlorate, chloride, perchlorate	0.5 m*M* cerium(III) sulfate, 0.5 m*M* 2,5-dihydroxybenzoic acid, pH=3.4	251, 345	n/a	standard solutions	method for the simultaneous determination of anions and cations	[104]
3,5-dimethoxy benzoate	alpha cyclodextrin, beta cyclodextrin, gamma cyclodextrin	100 m*M* 3,5-dimethoxy benzoate, 100m*M* Tris, pH=7	254	50 μ*M*	fermentation broth, urine, plasma, pharmaceutical preparations	method for the analysis of cyclodextrins	[30]
3,4-dimethoxycinnamic acid	fructose, glucose, maltose, sucrose	12 m*M* 3,4-dimethoxycinnamic acid, 63 m*M* lithium hydroxide	310, 267, 256	0.01-0.04 m*M*	culture media	method for the analysis of sugars	[105]
(N,N'-dimethylamino)-benzoate	alanine, asparagine, aspartic acid, cysteine, glutamic acid, glutamine, glycine, histidine, iso leucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, valine	5 - 10 m*M* (N,N'-diemthylamino)benzoate, pH=10-11.2	222-288	n/a	standard solutions	investigation of nine probes for the analysis of amino acids	[98]
2,4-dimethyl benzoate	alpha cyclodextrin, beta cyclodextrin, gamma cyclodextrin	100 m*M* 2,4-dimethy benzoate, 100m*M* Tris, pH=7	254	50 μ*M*	fermentation broth, urine, plasma, pharmaceutical preparations	method for the analysis of cyclodextrins	[30]

See p. 15 for notes on organisation of this Table. See p. 67 for References. See p. 51 for abbreviations.

Table 1.2 (continued) Benzene Carboxylate Probes

Probe	Analytes	BGE	Detection Wavelength (nm)	LOD	Matrix	Comments	Ref.
2,5-dimethyl benzoate	alpha cyclodextrin, beta cyclodextrin, gamma cyclodextrin	100 m*M* 2,5-dimethyl benzoate, 100m*M* Tris, pH=7	254	50 μ*M*	fermentat-ion broth, urine, plasma, pharmaceutical preparations	method for the analysis of cyclodextrins	[30]
3,5-dimethyl benzoate	alpha cyclodextrin, beta cyclodextrin, gamma cyclodextrin	100 m*M* 3,5-dimethyl benzoate, 100m*M* Tris, pH=7	254	50 μ*M*	fermentat-ion broth, urine, plasma, pharmaceutical preparations	method for the analysis of cyclodextrins	[30]
3,5-dinitrobenzoate	caprylate, laurate, myristate, oleate, palmitate, stearate	5 m*M* 3,5-dinitrobenzoate, 10 m*M* phosphate, pH = 8.0, 50 % n-propanol	200, 254	6 μ*M*	human stratum corneum	method for the analysis of skin fatty acids	[28]
3,5-dinitrobenzoate	C1-C18 fatty acids	5 m*M* 3,5-dinitrobenzoic acid, 0.5 m*M* CTAB, 0-70% acetone pH=9.0	214, 254	n/a	fat hydrolysates of butter and palm oil	method for the analysis of homologous fatty acids	[106]
Hydroxybenzoate	acetate	4.0 m*M* hydroxybenzoate, 0.5 m*M* TTAB, pH=6.0	450, 220	0.1 μg/ml	antifungal lipopeptide	comparison of analysis of acetate in an antifungal lipopeptide by CE and IC	[107]
p-hydroxybenzoate	bromide, butanesulfonate, carbonate, chloride, citrate, ethanesulfonate, hexanesulfonate, molybdate, nitrate, nitrite, pentanesulfonate, phthalate, propanesulfonate, sulfate, tungstate	5.0 m*M* p-hydroxybenzoate, 0.5 m*M* TTAB, pH=9.37	254	n/a	standard solutions	method development strategies for analysis of anions	[96]

See p. 15 for notes on organisation of this Table. See p. 67 for References. See p. 51 for abbreviations.

Table 1.2 (continued) Benzene Carboxylate Probes

Probe	Analytes	BGE	Detection Wavelength (nm)	LOD	Matrix	Comments	Ref.
p-hydroxybenzoate	bromide, butanesulfonate, carbonate, chloride, citrate, ethanesulfonate, hexanesulfonate, molybdate, nitrate, nitrite, pentanesulfonate, phthalate, propanesulfonate, sulfate, tungstate	5.0 m*M* p-hydroxybenzoate, 0.5 m*M* TTAB, pH=9.37	254	n/a	standard solutions	method development strategies for analysis of anions	[96]
Phenylacetic acid	carbohydrates	0.1 m*M* phenylacetic acid, pH=12.2	222	0.1 m*M*	fruit juice, dairy products	analysis of carbohydrates; comparison of several probes	[108]
Phthalate	sulfate, sulfide, tetrathionate, thiosulfate	3 mM phthalate, 10 mM Tris, 0.5 mM DETA, pH=8.14	254, 214	2-4 μM	natural water	optimisation of BGE for the analysis of sulfur species	[29]
Phthalate	acetate, carbonate	5.0 m*M* phthalate, 4 m*M* sodium tetraborate, 0.5 m*M* TTAB, pH=5.88	254	12 μ*M*	O-acetylated polysaccharides	comparison of IC and CE for the determination of O-acetate groups in bacterial polysaccharides	[26]
Phthalate	bromide, chloride, citrate, fluoride, phosphate, sulfate	5 m*M* phthalate, 0.5 m*M* TTAB, pH=8.0	254	n/a	standard solutions	evaluation of BGE for low molecular mass anions	[2]
Phthalate	inorganic anions, alkyl sulfates, organic acids	5 m*M* phthalate, 0.5 m*M* TTAB, pH=5.6	254	n/a	Kraft black liquor, dental plaque extract, human saliva, butyric acid extract of air filter,	optimisation of analysis for anions in real samples	[69]
Phthalate	bisinositol phosphate, hexakisinositol phosphate, monoinositol phosphate, trisinositol phosphate	5.0 m*M* potassium hydrogen phthalate, 2.0 m*M* sodium tetraborate, 0.5 m*M* TTAB, pH=5.9	270, 254	200 ng/ml	physiological samples.	investigation of feasibility to analise for inositol phosphates by CE	[20]

See p. 15 for notes on organisation of this Table. See p. 67 for References. See p. 51 for abbreviations.

Table 1.2 (continued) Benzene Carboxylate Probes

Probe	Analytes	BGE	Detection Wavelength (nm)	LOD	Matrix	Comments	Ref.
Phthalate	acetate, ascorbate, citrate, glucanate, glutamate, lactate, malate, oxalate, tartrate	5 m*M* phthalate, 0.5 m*M* TTAB, pH=8.0	220, 240, 265	n/a	beverages	investigation of suitable probes for the analysis of organic acids	[68]
Phthalate	acetate, formate, glycolate, malate, oxalate, succinate, tartrate	phthalate, 0.4 m*M* TTAB, 0.6 m*M* calcium, pH=5.6	254	n/a	chicory root juices	method for the analysis of organic acids in sugar refinery juices	[109]
Phthalate	bromoacetate, chloroacetate, formate, monofluoroacetate	5 m*M* phthalate, 0.3 m*M* CTAB, pH=4.61	230	0.1-0.4 µg/ml	rodenticide baits	method for the determination of monofluoroacetate in rodenticide baits	[110]
Phthalate.	acetate, bromide, butyrate, chloride, formate, nitrate, nitrite, oxalate, propionate, sulfate	5.0 m*M* phthalate, 0.5 m*M* TTAB, 1.0 m*M* boric acid, pH=5.9	254, 280	100-200 ng/ml	atmospheric aerosols	comparison of probes for the analysis of organic and inorganic anions; comparison of CE and IC.	[38]
Phthalate	adipate, alpha-ketoglutarate, citrate, ethylmalonate, glutarate, lactate, malonate, methylmalonate, methylsuccinate, oxalate, pyruvate, succinate, tartrate	5 m*M* carbonate, 1.5 m*M* phthalate, 0.15 m*M* MTAB, pH=9.0	230	60-360 pg	human serum and urine	method for the analysis of biologically important organic acids	[111]
Phthalate	acetate, formate, lactate	5 m*M* phthalate, 1 m*M* TTAB, pH=5.6	254	n/a	cigarette smoke	determination of organic acids in cigarette smoke by HPLC and CE	[112]
Phthalate	acetate, citraconate, citrate, crotonate, hydroxyisobutyrate, itaconate, mesaconate, methacrylate, pyruvate	5 m*M* potassium hydrogen phthalate, 0.001% polybrene, pH=3.85	185	0.7-1.1 mg/l	supercritical water reaction broths of citric and itaconic acid	methods for the analysis of organic acids by both direct and indirect UV detection	[113]
Phthalate	aminomethylphosphonate, glyphosphate	10 m*M* phthalate, 0.5 m*M* TTAB, pH=7.5	240	9-12 µ*M*	milled wheat	optimisation of method for the analysis of glyphosphate and aminomethylphosphonate	[114]

See p. 15 for notes on organisation of this Table. See p. 67 for References. See p. 51 for abbreviations.

Table 1.2 (continued) Benzene Carboxylate Probes

Probe	Analytes	BGE	Detection Wavelength (nm)	LOD	Matrix	Comments	Ref.
Phthalate	phosphate, pyrophosphate, tripolyphosphate	5 m*M* potassium hydrogen phthalate, 0.5 m*M* DTAB, pH=4.2	250	n/a	potato bathes	method for the analysis of condensed phosphate; comparison of results with IC	[115]
Phthalate	bromide, chloride, nitrate, sulfate	0.02 *M* phthalate pH=6.5	228	0.3 -1.1 fmol	standard solutions	strategies to optimise indirect UV detection of anions	[46]
Phthalate	azide, bromide, chloride, fluoride, iodide, nitrate, nitrite, oxalate, sulfate, thiocyanate, thiosulfate	1 m*M* phthalate, 2 m*M* dimethylformamide, 2% water in methanol	254	30 μ*M*	standard solutions	analysis of inorganic anions in non-aqueous media	[116]
Phthalate	acetate, citrate, diphosphonic acid, maleate, succinate, tartrate	5.0 m*M* phthalate, 50 m*M* MES, 0.5 m*M* TTAB, pH=5.2	254	1 mg/l	acid counter-ions of basic drugs	optimisation of method for analysis of organic acid counter ions of basic drugs	[117]
Phthalate	farnesyl pyrophosphate, geranyl pyrophosphate, geranylgeranyl pyrophosphate, isopentenyl pyrophosphate	2.5 -5.0 m*M* phthalate, pH=6.5	228	0.5 μ*M*	standard solutions, rat liver	comparison of direct UV, indirect UV, and indirect fluorescence detection for the analysis of isoprenyl pyrophosphates	[118]
Phthalate	bromate, chlorate, chloride, lactate succinate, malate, nitrate, nitrite, oxalate, sulfate, tartrate	5 m*M* phthalate, 5 m*M* TTAB, 50 m*M* MES, pH=5.2	205	n/a	coffee	method for the analysis of inorganic anions and organic acids	[119]
Pyromellitate	acetate, ascorbate, citrate, glucanate, glutamate, lactate, malate, oxalate, tartrate	2 m*M* pyromellitate, 2 m*M* DETA, pH=9.0	220, 240, 265	n/a	beverages	investigation of suitable probes for the analysis of organic acids	[68]
Pyromellitate	bromate, chlorate, chloride, lactate succinate, malate, nitrate, nitrite, oxalate, sulfate, tartrate	3 m*M* pyromellitic acid, 0.02% diethylenetriamine, 1% methanol, pH=9.6	205	n/a	coffee	method for the analysis of inorganic anions and organic acids	[119]

See p. 15 for notes on organisation of this Table. See p. 67 for References. See p. 51 for abbreviations.

Table 1.2 (continued) Benzene Carboxylate Probes

Probe	Analytes	BGE	Detection Wavelength (nm)	LOD	Matrix	Comments	Ref.
Pyromellitate	sulfate, sulfide, tetrathionate, thiosulfate	1.5 m*M* pyromellitate, 10 m*M* Tris, 0.5 m*M* DETA, pH=8.15	254, 214	2-4 μ*M*	natural water	optimisation of BGE for the analysis of sulfur species	[29]
Pyromellitate	arsenate, bromate, bromide, chlorate, chloride, citrate, fluoride, formate, malate, malonate, molybdate, nitrate, nitrite, oxalate, perchlorate, phosphate, sulfate, sulfosuccinate, tartrate, tetrafluoroborate, thiocyanate, thiosulfate, tungstate	2.25 m*M* pyromellitate, 6.5 m*M* sodium hydroxide, 1.6 m*M* TEA, 0.75 m*M* HMB, pH=7.7	250	0.2-1.0 ng/ml emi	silicon wafer surfaces	optimisation of electrokinetic sample introduction for the analysis of anions on silicon wafer surfaces	[40]
Pyromellitate	bromide, chloride, citrate, fluoride, phosphate, sulfate	5 m*M* pyromellitate, 0.5 m*M* TTAB, pH=8.0	254	n/a	standard solutions	evaluation of BGE for low molecular mass anions	[2]
Pyromellitate	bromide, chloride, fluoride, nitrate, nitrite, phosphate, sulfate, thiosulfate	2.25 m*M* pyromellitate, 6.5 m*M* sodium hydroxide, 1.6 m*M* TEA, 0.75 m*M* HMB pH=7.7	254	1-3 mg/l	standard solutions	validation of CE method for the analysis of anions	[39]

See p. 15 for notes on organisation of this Table. See p. 67 for References. See p. 51 for abbreviations.

Table 1.2 (continued) Benzene Carboxylate Probes

Probe	Analytes	BGE	Detection Wavelength (nm)	LOD	Matrix	Comments	Ref.
Pyromellitate	azide, bromate, bromide, butanesulfonate, chlorate thiocyanate, chloride, dichloroacetate, difluoroacetate, ethanesulfonate, fluoride, formate, heptanesulfonate, hexanesulfate, methanesulfonate, molybdate, monobromoacetate, monochloroacetate, nitrate, nitrite, octanesulfonate, pentanesulfonate, phosphate, phthalate, propanesulfonate, sulfate, thiosulfate, tribromoacetate, trichloroacetate, trifluoroacetate	2.5 m*M* pyromellitic acid, 0.75 HMB, pH=3.5 with NaOH 2.5 m*M* pyromellitic acid, 6.5 m*M* NaOH, 0.75 m*M* HMB, 1.6 m*M* TEA	250	n/a	standard solutions	investigations of parameters which effect the separation and detection of anions	[41]
Pyromellitate	acetate, chloride, citrate, lactate, malate, nitrate, oxalate, phosphate, succinate, sulfate, tartrate	3 m*M* pyromellitate, 3 m*M* DETA, pH=7.5	220	0.01-1.0 mg/l 3-300 fmol	red and white wine, apple juice	optimisation of method for analysis of inorganic and organic acids in food and beverages	[120]
Pyromellitate	acetate, bromide, butyrate, chloride, formate, nitrate, nitrite, oxalate, propionate, sulfate	2.25 pyromellitate, 0.75 HMB, 6.5 m*M* NaOH, 1.60 m*M* TEA pH=7.7	254, 280	100-200 ng/ml	atmospheric aerosols	comparison of probes for the analysis of organic and inorganic anions; comparison of CE and IC	[38]
Pyromellitate	bromide, chloride, fluoride, nitrate, phosphate, sulfate	2.25 m*M* pyromellitate, 1.6 m*M* TEA, pH=7.9	250	n/a	standard solutions	investigation of coating fused silica capillaries with polyamide resin to provide a positive capillary surface	[121]

See p. 15 for notes on organisation of this Table. See p. 67 for References. See p. 51 for abbreviations.

Table 1.2 (continued) Benzene Carboxylate Probes

Probe	Analytes	BGE	Detection Wavelength (nm)	LOD	Matrix	Comments	Ref.
Pyromellitate	chloride, fluoride, nitrate, phosphate, sulfate	2.25 pyromellitate, 6.5 m*M* sodium hydroxide, 1.6 m*M* TEA, 0.75 m*M* HMB, pH=7.7	215	1-14 µg/l emi	standard solutions	investigation of factors influencing trace ion analysis by electrostacking	[42]
Pyromellitate	bromide, chloride, nitrate, nitrite, oxalate, sulfate	2.25 m*M* pyromellitate, 6.5 m*M* NaOH, 0.75 m*M* HMOH, 1.6 m*M* TEA, pH=7.7-7.9	254	0.04-0.15 mg/l	aerosols	comparison of IC and CE for the analysis of inorganic anions in aerosols	[122]
Salicylate	ascorbate, carbonate, chloride, citrate, formiate, fumarate, glutarate, lactate, malate, nitrate, oxalate, phosphate, pyruvate, succinate, sulfate	7.5 m*M* salicylic acid, 15 m*M* Tris, 1 m*M* calcium hydroxide, 0.5 m*M* DoTAOH	232	0.2-2 µ*M*	plants	method for the analysis of inorganic anions and carboxylic acids in plant matrices	[36]
Salicylate	alanine, glutamic acid, glycine, leucine, proline, serine, valine	5 m*M* sodium salicylate, pH=11.0	n/a	n/a	standard solutions	theoretical considerations of indirect detection	[6]
Salicylate	alanine, asparagine, aspartic acid, cysteine, glutamic acid, glutamine, glycine, histidine, iso leucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, valine	5 – 10 m*M* salicylate, pH=10-11.2	222-288	n/a	standard solutions	investigation of nine probes for the analysis of amino acids	[98]
Salicylate	alkane sulfonates, alkyl sulfates	5.0 m*M* acetate, 5.0 m*M* salicylate, pH=6.0 + 20 m*M* monovalent cation or 1.0 m*M* alkaline earths	230	0.6 pmol	anionic detergents.	study of the effect of addition of cations to BGEs on resolution of anionic surfactants	[123]
Salicylate	cyclodextrins, methyl-, dimethyl-, trimethyl-, hydroxypropyl derivatives of beta cyclodextrin	50 m*M* salicylic acid, pH=6.0	220, 224	n/a	standard solutions	analysis of cyclodextrins and determination of formation constants for inclusion complexes	[124]

See p. 15 for notes on organisation of this Table. See p. 67 for References. See p. 51 for abbreviations.

Table 1.2 (continued) Benzene Carboxylate Probes

Probe	Analytes	BGE	Detection Wavelength (nm)	LOD	Matrix	Comments	Ref.
2-sulfobenzoic acid	bromide, chloride, nitrate, sulfate	0.02 *M* 2-sulfobenzoic acid, pH=6.5	228	0.3 -1.1 fmol	standard solutions	strategies to optimise indirect UV detection of anions	[46]
5-sulfosalicylic acid	heparin fragments	5 *mM* 5-sulfosalicylic acid pH=3.0	214	5 fmol	synthetic heparin fragments	investigation of analysis of heparin fragments	[125]
Terephthalic acid	acetate, ascorbate, citrate, glucanate, glutamate, lactate, malate, oxalate, tartrate	5 m*M* terephthalate, 0.25 m*M* TTAB, pH=9.0	220, 240, 265	n/a	beverages	investigation of suitable probes for the analysis of organic acids	[68]
1,2,4-tricarboxybenzoic acid	heparin fragments	5 m*M* 1,2,4-tricarboxybenzoic acid, pH=3.5	214	5 fmol	synthetic heparin fragments	investigation of analysis of heparin fragments	[125]
Trimellitate	acetate, ascorbate, citrate, glucanate, glutamate, lactate, malate, oxalate, tartrate	5 m*M* trimellitate, 1 m*M* TTAB, pH=9.0	220, 240, 265	n/a	beverages	investigation of suitable probes for the analysis of organic acids	[68]
Trimellitate	bromide, chloride, citrate, fluoride, phosphate, sulfate	5 m*M* trimellitate, 0.5 m*M* TTAB, pH=8.0	254	n/a	standard solutions	evaluation of BGE for low molecular mass anions	[2]
Trimellitate	chloride, citrate, hexanoate, malonate, propionate, tartrate	1 m*M* trimellitate, Tris pH=8.09.	254	n/a	standard solutions	consideration of theory of displacement by analytes with binary buffers	[16]
Trimellitate	hexanoate, propionate	0.5 m*M* trimellitate, Tris pH=8.2	210	n/a	standard solutions	consideration of factors that effect limits of detection and noise of indirect detection	[37]
Trimellitate	chromium(III), cobalt(II), copper(I), gold, iron(II), iron(III), nickel(II), platinum, silver	2.5 m*M* trimellitate, 0.8 m*M* HMB, pH=9.5	254	n/a	standard solutions	method for the separation of metallo-cyanide complexes	[126]

See p. 15 for notes on organisation of this Table. See p. 67 for References. See p. 51 for abbreviations.

Table 1.2 (continued) Benzene Carboxylate Probes

Probe	Analytes	BGE	Detection Wavelength (nm)	LOD	Matrix	Comments	Ref.
Trimesic acid	bromide, carbonate, chlorate, chloride, fluoride, nitrate, nitrite, perchlorate, phosphate, sulfate	Anion analysis buffer (Perkin Elmer).	230	50 µg/l	waste water	determination of inorganic anions in waste water	[127]

See p. 15 for notes on organisation of this Table. See p. 67 for References. See p. 51 for abbreviations.

Table 1.3 Naphthalene Carboxylate Probes

Probe	Analytes	BGE	Detection Wavelength (nm)	LOD	Matrix	Comments	Ref.
2,6-naphthalenedicarboxylate	acetate, butyrate, propionate	1 m*M* 2,6-naphthalenedicarboxylate, 0-8.0 m*M* beta cyclodextrin, pH=9.0	240	0.025 mg/l	standard solutions	method optimisation of dynamic range for the analysis of small anions by manipulating the probe mobility with complexing agents	[128]
2,6-naphthalenedicarboxylate	acetate, adipate, azelate, benzoate, butyrate, carbonate, chloroacetate, dichloroacetate, formate, fumarate, glutarate, methanesulfonate, phthalate, pimelate, propionate, sebacate, suberate	2 m*M* 2,6-naphthalenedicarboxylate, 0.5 m*M* TTAB, pH=8,11 with NaOH	280	20 µg/ml	atmospheric aerosols	study on the suitability of 2,6-naphthalenedicarboxylate for use as a probe	[129]
2,6-naphthalenedicarboxylate	acetate, bromide, butyrate, chloride, formate, nitrate, nitrite, oxalate, propionate, sulfate	2 m*M* 2,6-naphthalenedicarboxylate, 0.5 m*M* TTAB, 5.0 m*M* NaOH, pH=10.9	254, 280	100-200 ng/ml	atmospheric aerosols	comparison of probes for the analysis of organic and inorganic anions; comparison of CE and IC	[38]
Naphthoate	C2-C14 fatty acids	10 m*M* naphthoate, Tris, 30%THF, pH=8.2	254, 270, 300	1-2.5 µ*M*	cocoa oil extract	comparison of probes for the analysis of C2-C14 frees fatty acids; method validation for anisate as probe for the analysis of cocoa oil extract	[31]
1-naphthylacetic acid	carbohydrates	0.1 - 2 m*M* naphthylacetic acid, pH=12.2	222	0.1 m*M*	fruit juice, dairy products	analysis of carbohydrates; comparison of several probes	[108]

See p. 15 for notes on organisation of this Table. See p. 67 for References. See p. 51 for abbreviations.

Table 1.3 (continued) Naphthalene Carboxylate Probes

Probe	Analytes	BGE	Detection Wavelength (nm)	LOD	Matrix	Comments	Ref.
1-naphthylacetic acid	cyclodextrins, methyl-, dimethyl-, trimethyl-, hydroxypropyl derivatives of beta cyclodextrin	2 m*M* 1-naphthylacetic acid, pH=12.2	220, 224	n/a	standard solutions	analysis of cyclodextrins and determination of formation constants for inclusion complexes	[124]
1-naphthylacetic acid	chloride, citrate, hexanoate, malonate, propionate, tartrate	0.25 mm naphthylacetic acid, Tris pH=8.09.	254	n/a	standard solutions	consideration of theory of displacement by analytes with binary buffers	[16]

See p. 15 for notes on organisation of this Table. See p. 67 for References. See p. 51 for abbreviations.

Table 1.4 Aromatic Sulfonate Probes

Probe	Analytes	BGE	Detection Wavelength (nm)	LOD	Matrix	Comments	Ref.
1,3-benzene-disulfonate	sulfate, sulfide, tetrathionate, thiosulfate	3 m*M* 1,3-benzene-disulfonate, 10 m*M* Tris, 0.5 m*M* DETA, pH=8.15	254, 214	2-4 μ*M*	natural water	optimisation of BGE for the analysis of sulfur species	[29]
1,5-naphthalene-disulfonate	1,2,6-inositol triphosphate	0.5 m*M* 1,5-naphthalene-disulfonate, 30 m*M* acetic acid, pH=3.0	214	n/a	plasma	method for the analysis of 1,2,6-inositol triphosphate in plasma	[21]
1,5-naphthalene-disulfonate	sulfate, sulfide, tetrathionate, thiosulfate	3 m*M* 1,5-naphthalene-disulfonate, 10 m*M* Tris, 0.5 m*M* DETA, pH=8.15	254, 214	2-4 μ*M*	natural water	optimisation of BGE for the analysis of sulfur species	[29]
1,5-naphthalene-disulfonate	Inorganic anions, organic acids, alkyl sulfates	4 - 8.3 m*M* 1,5-naphthalene-disulfonate, 100 m*M* boric acid, 5 m*M* borate, 2 m*M* DETA, pH=8	288, 284, 214	30-350 μg/ml	standard solutions	investigation of naphthalene sulfonates as probes for the analysis of inorganic anions, organic acids, and surfactants	[130]
Naphthalene-sulfonate	Inorganic anions, alkyl sulfates, organic acids	10 m*M* naphthalene-sulfonate, 30 % acetonitrile, pH=10.0	254	n/a	Kraft black liquor, dental plaque extract, human saliva, butyric acid extract of air filter,	optimisation of analysis for anions in real samples	[69]
2-naphthalene-sulfonate	C2-C14 fatty acids	10 m*M* 2-naphthalene-sulfonate, Tris, 30%THF, pH=8.2	254, 270, 300	1-2.5 μ*M*	cocoa oil extract	comparison of probes for the analysis of C2-C14 frees fatty acids; method validation for anisate as probe for the analysis of cocoa oil extract	[31]
2-naphthalene-sulfonate	anion and cation surfactants	5 m*M* benzylamine, 5 m*M* 2-naphthalene-sulfonate, 100 m*M* borate, pH=6.0, 50% methanol	220	n/a	standard solutions	simultaneous analysis of cations and anions by using UV absorbing counter and co ions	[24]

See p. 15 for notes on organisation of this Table. See p. 67 for References. See p. 51 for abbreviations.

Table 1.4 (continued) Aromatic Sulfonate Probes

Probe	Analytes	BGE	Detection Wavelength (nm)	LOD	Matrix	Comments	Ref.
2-naphthalene-sulfonate	alkyl sulfates	4 - 8.3 m*M* 2-naphthalene-sulfonate, 100 m*M* boric acid, 5 m*M* borate, 2 m*M* DETA, pH=8	288, 284, 214	30-350 µg/ml	standard solutions	investigation of naphthalene sulfonates as probes for the analysis of inorganic anions, organic acids, and surfactants	[130]
2-naphthalene-sulfonic acid	carbohydrates	0.1 m*M* 2-naphthalene-sulfonic acid, pH=12.2	222	0.1 m*M*	fruit juice, dairy products	analysis of carbohydrates; comparison of several probes	[108]
1,3,6-naphthalene-trisulfonate	sulfate, sulfide, tetrathionate, thiosulfate	2 m*M* 1,3,6-naphthalene-trisulfonate, 10 m*M* Tris, 0.5 m*M* DETA, pH=8.15	254, 214	2-4 µ*M*	natural water	optimisation of BGE for the analysis of sulfur species	[29]
1,3,6-naphthalene-trisulfonate	Inorganic anions, organic acids, alkane sulfonates, alkyl sulfates	4 - 8.3 m*M* 1,3,6-naphthalene-trisulfonate, 100 m*M* boric acid, 5 m*M* borate, 2 m*M* DETA, pH=8	288, 284, 214	30-350 µg/ml	standard solutions	investigation of naphthalene sulfonates as probes for the analysis of inorganic anions, organic acids, and surfactants	[130]
1-naphthol-3,6-disulfonic acid	inositol phosphates	0.5 m*M* 1-naphthol-3,6-disulfonic acid, 30 m*M* acetic acid, 0.01% hydroxypropylmethyl-celluose	214	4-22 µ*M*	fermentation broths	determination of inositol phosphates in fermentation broth	[131]
1-nitroso-2-naphhthol-3,6-disulfonate	acetate, butyrate, chloride, clodronate, fluoride, hippurate, isovalerate, malonate, nitrate, nitrite, oxalate, phosphate, phosphite, propionate, pyruvate, sulfate	0.5 m*M* 1-nitroso-2-naphthol-3,6-disulfonate, pH=8	254	$2x10^{-8}$ - $9x10^{-7}$ *M*	standard solutions	method for the analysis of small anions using sulfonated nitrosonaphthol dyes	[132]

See p. 15 for notes on organisation of this Table. See p. 67 for References. See p. 51 for abbreviations.

Table 1.4 (continued) Aromatic Sulfonate Probes

Probe	Analytes	BGE	Detection Wavelength (nm)	LOD	Matrix	Comments	Ref.
1-nitroso-2-naphthol-6-sulfonate	acetate, butyrate, chloride, clodronate, fluoride, hippurate, isovalerate, malonate, nitrate, nitrite, oxalate, phosphate, phosphite, propionate, pyruvate, sulfate	0.5 m*M* 1-nitroso-2-naphthol-6-sulfonate, pH=8	254	$2x10^{-8}$ - $9x10^{-7}$ *M*	standard solutions	method for the analysis of small anions using sulfonated nitrosonaphthol dyes	[132]
2-nitroso-1-naphthol-6-sulfonate	acetate, butyrate, chloride, clodronate, fluoride, hippurate, isovalerate, malonate, nitrate, nitrite, oxalate, phosphate, phosphite, propionate, pyruvate, sulfate	0.5 m*M* 2-nitroso-1-naphthol-6-sulfonate, pH=8	254	$2x10^{-8}$ - $9x10^{-7}$ *M*	standard solutions	method for the analysis of small anions using sulfonated nitrosonaphthol dyes	[132]
p-toluenesulfonate	anion and cation surfactants	5 m*M* pyridine, 5 m*M* p-toluenesulfonate, 0% and 50 % methanol, 100 m*M* borate, pH = 6.0 5 m*M* benzylamine, 5 m*M* p-toluenesulfonic acid, 100 m*M* borate, pH=6.0, 50% methanol	220	n/a	standard solutions	simultaneous analysis of cations and anions by using UV absorbing counter and co ions	[24]
trinitrobenzene-sulfonic acid	C1-C18 fatty acids	10 m*M* trinitrobenzene-sulfonic acid, 60% acetonitrile, pH=9.0	214, 254	n/a	fat hydrolysates of butter and palm oil	Method for the analysis of homologous fatty acids.	[106]

See p. 15 for notes on organisation of this Table. See p. 67 for References. See p. 51 for abbreviations.

Table 1.5 Fluorescent Probes

Probe	Analytes	BGE	Detection Wavelength (nm)	LOD	Matrix	Comments	Ref.
Coumarin	d-arabinose, d-fructose, d-glucose, d-xylose, lactose, maltose, raffinose, sucrose	1 m*M* coumarin, pH=11.65	Ex: 488 Em: 640	2 fmol	standard solutions	indirect fluorescence detection of sugars	[133]
2,5-dihydroxybenzoic acid	bromate, carbonate, chloride, methanesulfonate, nitrate, oxalate, sulfate	1 m*M* 2,5-dihydroxybenzoic acid, 0.5 m*M* lead acetate.	Ex: 314 Em: 389	0.2 - 0.5 μ*M*	standard solutions	indirect fluorescence for the analysis of anions; investigation of selectivity changes when lead is added to the BGE	[134]
Fluorescein	dansylated amino acids	0.2 mg/ml fluorescein, 10 m*M* sodium tetraborate, pH=9.0	Ex: 325 Em: 550	n/a	standard solutions	study of extended pathlengths with tanscolumn illumination	[135]
Fluorescein	alkyl sulfonates and carboxylic acids	0.01 m*M* fluorescein, 5 m*M* sodium tetraborate, pH=9.2	Ex: 488 Em: 520	700 ppb	standard solutions	indirect fluorescent detection of C1-C10 n alkane sulfonates and C1-C18 carboxylic acids	[136]
Fluorescein	2,4,6-trichlorophenol, 2,4-chlorophenol, 2,4-dimethylphenol, 2,4-dinitrophenol, 2-methyl-4,6-dinitrophenol, 2-nitrophenol, 4-chloro-3-methylphenol, 4-nitrophenol, pentachlorophenol	1 m*M* fluorescein, 15 m*M* sodium borate, pH=9.9	Ex: 488 Em: 520	< 1 μ*M*	industrial waste waters	indirect fluorescence detection of phenols	[137]
Fluorescein	chloride, cyanate, cyanide, nitrate, sulfate, thiocyanate	0.1 m*M* fluorescein, pH=10	Ex: 488, Em: 500	5 μ*M*	alkaline oxidation synthetic sample	method for the analysis of cyanide by indirect fluorescence detection	[138]
Fluorescein	acetate, benzoate, chlorate, glutamate, iodide, lactate, nitrate, pyruvate	0.1 m*M* fluorescein, 0.5 mm Tris, pH=8.5	Ex: 330 Em: 400	$1x10^{-16}$ mol	erythrocytes	method for indirect fluorescence determination of lactate and pyruvate in single erythrocytes	[139]

See p. 15 for notes on organisation of this Table. See p. 67 for References. See p. 51 for abbreviations.

Table 1.5 (continued) Fluorescent Probes

Probe	Analytes	BGE	Detection Wavelength (nm)	LOD	Matrix	Comments	Ref.
Salicylate	adenosine, adenosine 5'-diphosphate, adenosine 5'-monophosphate, cytidene, cytidene 5'-diphosphate, cytidene 5'-monophosphate, guanosine 5'-diphosphate, guanosine 5'-monophosphate, thymidene 5'-diphosphate, thymidene 5'-monophosphate	250 μ*M* salicylate, pH=3.5	Ex: 325 Em: 405	70 amol	standard solutions	optimisation of Laser-induced indirect fluorescence detection sensitivity and separation	[140]
Salicylate	farnesyl pyrophosphate, geranyl pyrophosphate, geranylgeranyl pyrophosphate, isopentenyl pyrophosphate	1m*M* salicylic acid, pH=4.0, 5.0, 6.5	228 Ex: 325 Em: -	0.5 μ*M*	standard solutions, rat liver	comparison of direct UV, indirect UV, and indirect fluorescence detection for the analysis of isoprenyl pyrophosphates	[118]
Salicylate	alanine, arginine, aspartate, cysteine, glutamic acid, leucine, phenylalanine, proline, serine, tyrosine	200 μ*M* salicylate, 40 μ*M* carbonate, pH=9.7	Ex:325 Em: 405	10 μ*M*	standard solutions	indirect laser induced fluorescence detection of native amino acids	[141]
Salicylate	inorganic anions and nucleotides	1.0 m*M* salicylate, pH=3.5	Ex: 331 Em: -	600 amole	standard solutions	indirect fluorescence detection of inorganic anions and nucleotides	[142]
Salicylate	anionic peptides from tryptic digests of bovine serum albumin and beta casein	500 μ*M* salicylate, CAPS, pH=10.9	Ex: 330 Em: -	550 amol	tryptic digests of bovine serum albumin and beta casein	indirect fluorimetric detection of tryptic digests	[143]
Terbium(III)-acetylacetone	chromate, hexacyanoferrate(III), nitrite	0.8 m*M* acetylacetone, 0.35 m*M* terbium (III) chloride, 6 m*M* sodium chloride, pH=8.3	Ex: 295 Em: 545	0.1 μ*M*	standard solutions	investigation of using indirect luminescence detection	[144]

See p. 15 for notes on organisation of this Table. See p. 67 for References. See p. 51 for abbreviations.

Table 1.5 (continued) Fluorescent Probes

Probe	Analytes	BGE	Detection Wavelength (nm)	LOD	Matrix	Comments	Ref.
2-p-toluidino-naphthalene-6-sulfonate	dodecyl sulfate	0.1 m*M* 2-p-toluidino-naphthalene-6-sulfonate, 10 m*M* sodium hydrogen phosphate, 6.0 m*M* sodium tetraborate	Ex: 325 Em: 450	n/a	soap	indirect fluorescence detection of SDS in soap	[145]

See p. 15 for notes on organisation of this Table. See p. 67 for References. See p. 51 for abbreviations.

Table 1.6 Miscellaneous Probes

Probe	Analytes	BGE	Detection Wavelength (nm)	LOD	Matrix	Comments	Ref.
Adenosine monophosphate	polyphosphates and polyphosphonates	5m*M* adenosine monophosphate, 5 m*M* sodium tetraborate, 100 m*M* borate, 2 m*M* DETA, pH 7.8	259, 261	1.2 - 11 μ*M*	toothpaste, commercial herbicide	evaluation of ribonucleotide probes for the analysis of polyphosphates and polyphosphonates	[23]
Adenosine monophosphate	alkyl sulfates and sulfonates	5 m*M* adenosine monophosphate, 100 m*M* H_3BO_3, 50 % MeOH, pH=6.0	259, 254, 280, 300	0.05 - 3.0 mg/l	standard solutions	method for the analysis of anionic and cationic surfactants	[146]
Bromocresol green	pyruvate	0.5 m*M* bromocresol green pH=8.0	633	2x 10^{-16} mol	standard solutions	investigation of a double beam laser for indirect absorbance detection	[45]
Chlorophenol red	bromide, chloride, citrate, fumarate, glutamate, malonate, nitrate, sulfate	0.5 m*M* chlorophenol red., 5 m*M* Tris, pH=6.0 with acetic acid	578, 620, 635	2 x10^{-13} - 8x 10^{-16} mol	standard solutions	investigation of dyes as probes	[52]
p-cresol	carbohydrates	0.1 m*M* p-cresol, pH=12.2	222	0.1 m*M*	fruit juice, dairy products	analysis of carbohydrates; comparison of several probes	[108]
Diethylbarbiturate	fatty acids	5 m*M* diethylbarbiturate, 0.5 *M* Z1-Methyl, 70% ethylenemonomethylether, pH=10.5	240	n/a	butter	method for the analysis of free fatty acids	[147]

See p. 15 for notes on organisation of this Table. See p. 67 for References. See p. 51 for abbreviations.

Table 1.6 (continued) Miscellaneous Probes

Probe	Analytes	BGE	Detection Wavelength (nm)	LOD	Matrix	Comments	Ref.
Diethylbarbiturate	acetate, fatty acids, butyrate, formate, lactate, malonate, propionate	5m*M* diethylbarbiturate, 0.5 *M* Z1-Methyl, 70% ethyleneglycol monomethyl ether	240, 254	n/a	butter, standard solutions	review of detection methods and optimisation of selectivity using complexing reagents for the analysis of low molecular mass organic acids	[148]
1,3-dihydroxy-naphthalene	carbohydrates	0.1 m*M* 1,3-dihydroxy-naphthalene, pH=12.2	222	0.1 m*M*	fruit juice, dairy products	analysis of carbohydrates; comparison of several probes	[108]
Histidine	acetate, benzoate, chloride formate nitrate	20 m*M* histidine, 0.5m*M* butyric acid, 0.5 m*M* hydrochloric acid, pH=6.1	214	n/a	standard solutions	theoretical considerations of sample zones for weak acids	[14]
Indigo carmine	bromide, chloride, citrate, fumarate, glutamate, malonate, nitrate, sulfate	0.5 m*M* indigo carmine., 5 m*M* Tris, pH=6.0 with acetic acid	578, 620, 635	$2x10^{-13}$ -$8x10^{-16}$ mol	standard solutions	Investigation of dyes as probes.	[52]
Iodide	bromide, chloride, nitrate, nitrite, sulfate, thiosulfate	7.5 m*M* sodium iodide	254, 226, 270	n/a	standard solutions	evaluation of coated capillaries for the analysis of fast anions without the addition of EOF modifiers	[95]
Nicotinoate	alanine, asparagine, aspartic acid, cysteine, glutamic acid, glutamine, glycine, histidine, iso leucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, valine	5 - 10 m*M* nicotinoate, pH=10-11.2	222-288	n/a	standard solutions	investigation of nine probes for the analysis of amino acids	[98]

See p. 15 for notes on organisation of this Table. See p. 67 for References. See p. 51 for abbreviations.

Table 1.6 (continued) Miscellaneous Probes

Probe	Analytes	BGE	Detection Wavelength (nm)	LOD	Matrix	Comments	Ref.
Permanganate	chlorate, chloride, fluoride, nitrate, sulfate	20 m*M* permanganate, pH=7.0	635, 670	3.5 μ*M*	standard solutions	evaluation of double beam absorption systems based on light emitting diodes	[97]
Phenylphosphonic acid	butylphosphonic acid, ethylphosphonic acid, methylphosphonic acid, propylphosphonic acid	10 m*M* phenylphosphonic acid, 200 m*M* sodium borate, pH=6.0	200	0.15 - 0.21 pmol	standard solutions	method for the analysis of alkylphosphonic acids	[25]
2,6-pyridine-dicarboxylate	acetate, bromide, butyrate, citrate, formate, heptanoate, hexanoate, iodide, lactate, malate, nitrate, octanoate, oxalate, pyruvate, succinate, tartrate, valerate	5 m*M* 2,6-pyridine-dicarboxylate, 0.5 m*M* CTAB, pH=5.65	214	1-2.5 mg/l	beer	investigation of 2,6-pyridinedicarboxylic acid as probe; investigation of electrolyte's complexing ability to prevent adsorption of selected organic acids onto metal impurities	[149]
Riboflavin	fructose, glucose, maltose, sucrose	12 m*M* riboflavin acid, 63 m*M* lithium hydroxide	310, 267, 256	0.01 to 0.04 m*M*	culture media	method for the analysis of sugars	[105]
Sorbate	d-fucose, d-galactose, d-glucose, d-glucuronic acid galacturonate, N-acetyl-galactosamine, N-acetyl-glucosamine, N-acetyl-neuraminic acid	6 m*M* sorbate, pH=12.2	256	0.23-0.29 m*M*	fruit juice	comparison of HPLC and CE for the analysis of carbohydrates in fruit juices	[150]
Sorbate	carbohydrates	6 m*M* sorbic acid, pH=12.1	256	2 pmol	orange juice	method for the analysis of carbohydrates	[151]

See p. 15 for notes on organisation of this Table. See p. 67 for References. See p. 51 for abbreviations.

Table 1.6 (continued) Miscellaneous Probes

Probe	Analytes	BGE	Detection Wavelength (nm)	LOD	Matrix	Comments	Ref.
Sorbate	2-deoxy-d-galactose, 2-deoxy-d-ribose, cellobiose, d-arabonic acid, d-fructose, d-fucose, d-galactonic acid, d-galactose, d-galacturonic acid, d-gluconic acid, d-glucose, d-glucuronic acid, d-lyxose, d-mannonic acid, d-mannose, d-mannuronic acid, d-ribonic acid, d-ribose, d-xylose, lactose, lactulose, l-arabinose, l-rhamnose, l-sorbose, maltose, maltotriose, melibiose, raffinose, saccharose	6 m*M* sorbate, pH=12.1	254	2000 fmol	standard solutions	comparison of carbohydrate analysis by indirect and direct UV	[152]
Sorbate	ethylphosphonic acid, methylphosphonic acid, propylphosphonic acid, t-butylphosphonic acid	5 m*M* sorbate, 0.5 m*M* TTAB, pH=6.8	254	n/a	standard solutions	analysis of organophosphonic acids with borate esterification	[153]
Sorbate	fructose, glucose, maltose, sucrose	12 m*M* sorbate acid, 63 m*M* lithium hydroxide	310, 267, 256	0.01 - 0.04 m*M*	culture media	method for the analysis of sugars	[105]
Sorbate	aspartate, butyrate, chlorate, chloride, dichloroacetate, dimethylmalonate, glucuronate, glutamate, hydroxyisobutyrate, lactate, malonate, methylmalonate, phosphate	0.7 m*M* sorbic acid, histidine, pH=6.2	254	n/a	standard solutions	theoretical considerations of indirect detection, with some practical examples	[51]

See p. 15 for notes on organisation of this Table. See p. 67 for References. See p. 51 for abbreviations.

Table 1.6 (continued) Miscellaneous Probes

Probe	Analytes	BGE	Detection Wavelength (nm)	LOD	Matrix	Comments	Ref.
Sorbate	bromide, butanesulfonate, carbonate, chloride, citrate, ethanesulfonate, hexanesulfonate, molybdate, nitrate, nitrite, pentanesulfonate, phthalate, propanesulfonate, sulfate, tungstate	5 m*M* sorbate, 0.5 m*M* TTAB, pH=7.64	254	n/a	standard solutions	method development strategies for analysis of anions	[96]
Sorbate	alanine, asparagine, aspartic acid, cysteine, glutamic acid, glutamine, glycine, histidine, iso leucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, valine	5 – 10 m*M* sorbate, pH=10-11.2	222-288	n/a	standard solutions	investigation of nine probes for the analysis of amino acids	[98]
Sorbate	cyclodextrins, methyl-, dimethyl-, trimethyl-, hydroxypropyl derivatives of beta cyclodextrin	5 m*M* sorbate, pH=12.2	220, 224	n/a	standard solutions	analysis of cyclodextrins and determination of formation constants for inclusion complexes	[124]
Tiron	chloride, fluoride, iodide, nitrate, perchlorate, sulfate	5 m*M* tiron	293	n/a	sea water, standard solutions	considerations of influencing migration time for cations and anions	[154]

See p. 15 for notes on organisation of this Table. See p. 67 for References. See p. 51 for abbreviations.

Table 1.6 (continued) Miscellaneous Probes

Probe	Analytes	BGE	Detection Wavelength (nm)	LOD	Matrix	Comments	Ref.
Tryptophan	1-naphthylacetic acid, anthraquinone-2-sulfonic acid, cellobiose, d-arabinose, d-fructose, d-fucose, d-galactonic acid, d-galactosamine, d-galactose, d-gluconic acid, d-glucosamine, d-glucose, d-lyxose, d-mannitol, d-mannose, d-ribose, d-sorbitol, d-sorbose, d-tagatose, d-xylose, furanarcrylic acid, gentiobiose, lactose, maltose, meleziose, melibiose, muconic acid, raffinose, sorbate, stachyose, sucrose, tryptophan	1.0 m*M* tryptophan, 50 m*M* NaOH	280	n/a	standard solutions	analysis of carbohydrates using anionic and cationic probes	[155]
uridine monophosphate	polyphosphates and polyphosphonates	5m*M* uridine monophosphate, 5 m*M* sodium tetraborate, 100 m*M* borate, 2 m*M* DETA, pH 7.8	259, 261	1.2 -11 μ*M*	toothpaste, commercial herbicide	evaluation of ribonucleotide probes for the analysis of polyphosphates and polyphosphonates	[23]
Vanadate	bromide, chloride, nitrate, nitrite, sulfate, thiosulfate	7.5 m*M* sodium vanadate	254, 226, 270	n/a	standard solutions	evaluation of coated capillaries for the analysis of fast anions without the addition of EOF modifiers	[95]

See p. 15 for notes on organisation of this Table. See p. 67 for References. See p. 51 for abbreviations.

Table Abbreviations

CAPS = [3-(cyclohexylamino)propanesulfonic acid], CTAB = cetyltrimethylammonium bromide, CTAOH = cetyltrimethylammonium hydroxide, DETA = diethylenetriamine, DMB = decamethonium bromide, DoTAOH = dodecyltrimethylammonium hydroxide, DTAB = dodecyltrimethylammonium bromide, emi = electromigration injection, HDMB = hexadimethrine bromide, HMB = hexamethonium bromide, HMOH = hexamethonium hydroxide, HPLC = High Performance Liquid Chromatography, HTAB = hexadecyltrimethylammonium bromide, IC = Ion Chromatography, MES = morpholinoethanesulfonic acid, MTAB = myristyltrimethylammonium bromide, Nice Pak-OFM = Waters proprietry EOF modifer, SDS = sodium dodecyl sulfate, TBAB = tetrabutylammonium bromide, TEA = triethanolamine, THF = tetrahydrofuran, Tris = tris(hydroxymethyl)aminoethane , TTAB = tetradecyltrimethylammonium bromide , TTAOH = tetradecyltrimethylammonium hydroxide, Z1-methyl = Waters proprietry zwitter ion.

Table abbreviations.

Table 1.7 Probe Data

Probe	Mobility			Molar Absorptivity				*pKa*		Cross Ref.
	$10^{-9}m^2V^{-1}s^{-1}$	pH	Ref.	$lmol^{-1}cm^{-1}$	nm	pH	Ref.		Ref.	
Adenosine diphosphate	n/a	n/a	n/a	9200	259	7.8	[23]	n/a	n/a	n/a
Adenosine monophosphate	n/a	n/a	n/a	9335	n/a	n/a	[23]	n/a	n/a	[146]
p-aminobenzoate	-28.5	11	[98]	n/a	n/a	n/a	n/a	4.94	[98]	[99]
p-aminosalicylate	-30.1	n/a	[98]	n/a	n/a	n/a	n/a	3.25	[98]	n/a
Anisate	-30	Inf Dil	[156]	n/a	n/a	n/a	n/a	n/a	n/a	[31]
Benzoate	(1) -26.7 (2) -33.6 (3) -29.0	(1) 6.5 (2) Inf Dil (3) 11	(1) [149] (2) [19] (3) [98]	(1) 44480 (2) 11900 (3) 809	(1) 194 (2) 228 (3) 254	(1) 6.5 (2) 6.5 (3) 8.0	(1) [149] (2) [46] (3) [2]	4.21	[149]	[3], [30-32], [51], [68-69], [100-102]
1,3-benzenedisulfonate	-54.3	8.05	[29]	9950	214	8.05	[29]	n/a	n/a	n/a
o-benzylbenzoic acid	n/a	n/a	n/a	19000	228	6.5	[46]	n/a	n/a	n/a

See p. 16 for notes on the organisation of this Table. See p. 67 for references.

Table 1.7 (Continued) Probe Data

Probe	Mobility			Molar Absorptivity				*pKa*		Cross Ref.
	$10^{-9}m^2V^{-1}s^{-1}$	pH	Ref.	$lmol^{-1}cm^{-1}$	nm	pH	Ref.		Ref.	
Chlorophenol red	-22.1	n/a	[52]	(1) 28000 (2) 33000	(1) 578 (2) 578	(1) 6.5 (2) 7.3	(1) [52] (2) [52]	n/a	n/a	[39]
Chromate	(1) -56.7 (2) -81	(1) 6.5 (2) Inf Dil	(1) [149] (2) [77]	(1) 2640 (2) 3180	(1) 254 (2) 254	(1) 8.1 (2) 8.0	(1) [29] (2) [2]	(1) -0.98 (2) 6.5	[149]	[20], [22], [27], [31], [34-35], [38], [55-61], [63], [64-74], [76], [78-96], [157]
p-cresol	-28.5	12.1	[108]	8320	236	12.1	[108]	10.26	[108]	n/a
Cytidine monophosphate	n/a	n/a	n/a	5640	271	7.8	[23]	n/a	n/a	n/a
1,3-dihydroxynaphthalene	-29.3	12.1	[108]	14680	256	12.1	[108]	(1) 9.37 (2) 10.9	[108]	[108]
2,3-dihydroxybenzoate	-33	Inf Dil	[31]	n/a	n/a	n/a	n/a	n/a	n/a	[103]
3,4-dimethoxycinnamic acid	n/a	n/a	n/a	27000	310	n/a	[105]	n/a	n/a	n/a
(N,N'-dimethylamino)benzoate	-2.49	11	[98]	n/a	n/a	n/a	n/a	6.03	[98]	n/a

See p. 16 for notes on the organisation of this Table. See p. 67 for references.

Table 1.7 (Continued) Probe Data

Probe	Mobility			Molar Absorptivity				*pKa*		Cross Ref.
	$10^{-9}m^2V^{-1}s^{-1}$	pH	Ref.	$Imol^{-1}cm^{-1}$	nm	pH	Ref.		Ref.	
Guanosine monophosphate	n/a	n/a	n/a	8600	254	7.8	[23]	n/a	n/a	n/a
p-hydroxybenzoate	n/a	n/a	n/a	10299	254	8	[2]	(1) 4.48 (2) 9.32	[68]	[28], [96], [107]
Indigo carmine	-40	n/a	[52]	12000	620	4.2-7.3	[52]	n/a	n/a	n/a
2,6-naphthalenedicarboxylate	n/a	n/a	n/a	(1) 7667 (2) 10020	(1) 254 (2) 280	n/a	[129]	n/a	n/a	[38], [128]
1,5-naphthalenedisulfonate	-48.1	8.1	[29]	31000	214	8.1	[29]	n/a	n/a	[21], [130]
2-naphthalenesulfonate	(1) -31.3 (2) -17.4	(1) Inf Dil (2) 6.0	(1) [31] (2) [24]	11520	206	6	[24]	n/a	n/a	[108], [130]
1,3,6-naphthalenetrisulfonate	-62	8	[29]	31600	214	8	[29]	n/a	n/a	[130]
1-naphthylacetic acid	(1) -26.3 (2) -24.8 (3) -25.5	(1) 12.1 (2) 12.1 (3) 7.25	(1) [108] (2) [155] (3) [16]	81100	222	12.1	[108]	4.24	[108]	n/a

See p. 16 for notes on the organisation of this Table. See p. 67 for references.

Table 1.7 (Continued) Probe Data

Probe	Mobility			Molar Absorptivity				*pKa*		Cross Ref.
	$10^{-9}m^2V^{-1}s^{-1}$	pH	Ref.	$lmol^{-1}cm^{-1}$	nm	pH	Ref.		Ref.	
Nicotinoate	-30.9	11	[98]	n/a	n/a	n/a	n/a	4.82	[98]	n/a
Phenylacetic acid	-30	12.1	[108]	7600	209	12.1	[108]	4.31	[108]	n/a
Phthalate	(1)-48 (2) -41.2 (3) -55	(1) 8.1 (2) 6.5 (3) Inf Dil	(1) [29] (2) [149] (3) [77]	(1) 9950 (2) 37160 (3) 1357	(1) 214 (2) 196 (3) 254	(1) 8.1 (2) 6.5 (3) 8.0	(1) [29] (2) [149] (3) [2]	(1) 2.95 (2) 5.41	[149]	[20], [26], [38], [46], [68-69], [109-118]
Picrate	-31.5	Inf Dil	[31]	n/a	n/a	n/a	n/a	n/a	n/a	n/a
2,6-pyridinedicarboxylate	-41.5	6.5	[149]	43680	192	6.5	[149]	(1) 2.16 (2) 6.92	[149]	[149]
Pyromellitate	(1)-55.1 (2) 52.8	(1) 8.0 (2) 6.5	(1) [29] (2) [149]	(1) 26200 (2) 23900 (3) 7062	(1) 214 (2) 214 (2) 254	(1) 8.0 (2) 6.5 (3) 8.0	(1) [29] (2) [149] (3) [2]	(1) 1.92 (2) 2.87 (3) 4.49 (4) 5.63	[149]	[38-42], [68], [119-122]
Riboflavin	n/a	n/a	n/a	30000	267	n/a	[105]	n/a	n/a	n/a

See p. 16 for notes on the organisation of this Table. See p. 67 for references.

Table 1.7 (Continued) Probe Data

Probe	Mobility			Molar Absorptivity				*pKa*		Cross Ref.
	$10^{-9}m^2V^{-1}s^{-1}$	pH	Ref.	$lmol^{-1}cm^{-1}$	nm	pH	Ref.		Ref.	
Salicylate	(1) -37 (2) -32.9	(1) Inf Dil (2) 11	(1) [77] (2) [98]	n/a	n/a	n/a	n/a	2.94	[98]	[6], [28], [36], [118], [123-124], [140-143], [158]
Sorbate	(1) -29.6 (2) -28.9 (3) -28.1	(1) 12.1 (2) 12.1 (3) 11	(1) [108] (2) [155] (3) [98]	(1) 24120 (2) 28800	(1) 254 (2) 254	(1) 12.1 (2) 12.1	(1) [108] (2) [155]	4.77	[108]	[28], [51], [96], [105], [124], [150-153]
2-sulfobenzoic acid	n/a	n/a	n/a	40000	228	6.5	[46]	n/a	n/a	n/a
p-toluenesulfonate	-18.9	6	[24]	(1) 7520 (2) 344	(1) 221 (2) 254	(1) 6.0 (2) 8.0	(1) [24] (2) [2]	n/a	n/a	n/a
Trimellitate	(1) -47.3 (2) -63.9	(1) 6.5 (2) 7.25	(1) [149] (2) [16]	7147	254	8	[2]	(1) 2.52 (2) 3.84 (3) 5.20	[149]	[37], [68], [126]
Tryptophan	-21.8	12.1	[155]	5630	280	12.1	[155]	n/a	n/a	n/a
Uridine monophosphate	n/a	n/a	n/a	7240	261	7.8	[23]	n/a	n/a	n/a

See p. 16 for notes on the organisation of this Table. See p. 67 for references.

Table 1.8 Analyte Data

Analyte	Mobility			*pKa*		Cross Ref.
	$10^{-9}m^2V^{-1}s^{-1}$	pH	Ref.		Ref.	
Acetate	(1) -42.4 (2) -33.8	(1) Inf Dil (2) 6.5	(1) [14] (2) [149]	4.76	[149]	[3], [26], [38], [59], [60], [63], [65], [68-70], [79], [81], [85], [88], [93], [99], [101], [107], [109], [112-113], [117], [120] [128-129], [132], [139], [148]
N-acetyl-galactosamine	-6.3	12.1	[151]	n/a	n/a	[150]
N-acetyl-glucosamine	-8	12.1	[151]	n/a	n/a	[150]
N-acetyl-neuraminic acid	-19.9	12.1	[151]	n/a	n/a	[150]
Adenosine 5'-diphosphate	-23.79	3.5	[140]	n/a	n/a	[142]
Adenosine 5'-monophosphate	-8.28	3.5	[140]	n/a	n/a	n/a
Adipate	-43.5	Inf Dil	[159]	(1) 4.43 (2) 5.41	[111]	[129]
Alanine	-28	11	[6]	9.92	[6]	[98], [141]
d-arabinose	(1) -7.85 (2) -11.0	(1) 12.2 (2) 12.1	(1) [108] (2) [155]	12.43	[108]	[133]
l-arabinose	-5.1	12.1	[152]	n/a	n/a	[151]
d-arabonic acid	-27.6	12.1	[152]	n/a	n/a	[151]
Benzoate	(1) -33.6	Inf Dil	[14]	4.203	[14]	[14], [60], [63], [129], [139], [160]

See p. 16 for notes on the organisation of this Table. See p. 67 for references.

Table 1.8 (Continued) Analyte Data

Analyte	Mobility			*pKa*		Cross Ref.
	$10^{-9}m^2V^{-1}s^{-1}$	pH	Ref.		Ref.	
Bromide	-81	Inf Dil	[77]	n/a	n/a	[2], [38-41], [46], [52], [55], [60-61], [63-66], [69], [71], [73], [76], [79], [80], [82-83], [86-88], [90], [93], [95-96], [116], [121-122], [127], [130], [149]
Butyrate	(1) -33.8 (2) -26.7	(1) Inf Dil (2) 6.5	(1) [14] (2) [149]	4.82	[14]	[38], [51], [59], [60], [63], [65], [69], [79], [128-129], [132], [148]
C10-sulfate	-23.1	9.2	[136]	n/a	n/a	[130], [146]
Caprylate	11.6	8	[28]			[69]
Cellobiose	(1) -7.76 (2) -9.5 (3) -4.8	(1) 12.2 (2) 12.1 (3) 12.1	(1) [108] (2) [155] (3) [152]			[151-152]
Chlorate	-67	Inf Dil	[77]			[3], [40-41], [51], [57], [60], [63], [64-65],[81], [86-87], [93], [97], [104], [119], [127], [139], [142]

See p. 16 for notes on the organisation of this Table. See p. 67 for references.

Table 1.8 (Continued) Analyte Data

Analyte	Mobility			*pKa*		Cross Ref.
	$10^{-9}m^2V^{-1}s^{-1}$	pH	Ref.		Ref.	
Chloride	(1) -79.1 (2) -74.0	(1) Inf Dil (2) 8.08	(1) [14] (2) [16]	-2	[14]	[3], [35-36], [38-42], [46], [51-52], [55-56],[59-61], [63-67], [69-74], [76-77], [79-99], [104], [116], [119-122], [127], [130], [132], [134],[138], [154], [161]
Citrate	(1) -47.8 (2) -68 (3) -62.0	(1) 6.5 (2) 9 (3) 8.08	(1) [149] (2) [75] (3) [16]	(1) 3.13 (2) 4.76 (3) 6.40	[149]	[2], [36], [40], [52], [55], [57], [60], [63], [68], [79], [85], [96], [111], [113], [117], [120], [130]
Cytidene 5'-diphosphate	-21.79	3.5	[140]	n/a	n/a	[142]
Cytidene 5'-monophosphate	-2.31	3.5	[140],	n/a	n/a	[142]
2-deoxy-d-galactose	-2.42	12.1	[152]	n/a	n/a	[151]
2-deoxy-d-ribose	-2.8	12.1	[152]	n/a	n/a	[151]
Fluoride	-57	Inf Dil	[77]	n/a	n/a	[2-3], [35], [39-42], [55], [57], [60-61], [63-66], [69-72], [76], [79-80], [82], [84], [86], [87-88], [90], [93-94], [97], [116], [121], [127] [130], [132], [154]

See p. 16 for notes on the organisation of this Table. See p. 67 for references.

Table 1.8 (Continued) Analyte Data

Analyte	Mobility			*pKa*		Cross Ref.
	$10^{-9}m^2V^{-1}s^{-1}$	pH	Ref.		Ref.	
Formate	(1) -56.6 (2) -41.2	(1) Inf Dil (2) 6.5	(1) [14] (2) [149]	3.75	[149]	[3], [38], [40-41], [57], [59-60], [63], [65], [69-70], [79], [81], [101] [109-110], [112], [129-130], [148]
d-fructose	(1) -10.21 (2) -13.9 (3) -7.1	(1) 12.2 (2) 12.1 (3) 12.1	(1) [108] (2) [155] (3) [152]	12.03	[108]	[105], [133], [151]
d-fucose	-7.8	12.1	[155]	n/a	n/a	[150-152]
d-galactonic acid	(1) -24.5 (2) -25.0	(1) 12.1 (2) 12.1	(1) [155] (2) [152]	n/a	n/a	[151]
d-galactosamine	-9.5	12.1	[155]	n/a	n/a	[151]
d-galactose	(1) -6.86 (2) -9.5 (3) -4.5	(1) 12.2 (2) 12.1 (3) 12.1	(1) [108] (2) [155] (3) [152]	12.35	[108]	[150-151]
d-galacturonic acid	-26.8	12.1	[152]	n/a	n/a	n/a
gentiobiose	-9.5	12.1	[155]	n/a	n/a	n/a
d-gluconic acid	(1) -22.80 (2) -25.3 (3) -25.5	(1) 12.2 (2) 12.1 (3) 12.1	(1) [108] (2) [155] (3) [152]	3.86	[108]	[63], [151]
d-glucosamine	-11	12.1	[155]	n/a	n/a	[151]
d-glucose	(1) -8.04 (2) -11.0 (3) -5.1	(1) 12.2 (2) 12.1 (3) 12.1	(1) [108] (2) [155] (3) [152]	12.35	[108]	[105], [133], [150-151]
d-glucuronic acid	(1) -25.2 (2) -27.8	(1) 12.2 (2) 12.1	(1) [108] (2) [152]	n/a	n/a	[150-151]

See p. 16 for notes on the organisation of this Table. See p. 67 for references.

Table 1.8 (Continued) Analyte Data

Analyte	Mobility			*pKa*		Cross Ref.
	$10^{-9}m^2V^{-1}s^{-1}$	pH	Ref.		Ref.	
Glutamic acid	-44.3	11	[6]	9.96	[6]	[98], [141]
Glycine	-32.6	11	[6]	9.96	[6]	[98]
Guanosine 5'-diphosphate	-33.14	3.5	[140]	n/a	n/a	[142]
Guanosine 5'-monophosphate	-16.94	3.5	[140]	n/a	n/a	[142]
Heptanoate	-21.9	6.5	[149]	n/a	n/a	n/a
Hexanesulfate	-26.6	9.2	[136]	n/a	n/a	[41], [69], [146]
Hexanesulfonate	-28	Inf Dil	[146]	n/a	n/a	[96], [136]
Hexanoate	(1) -23.2 (2) -30.2 (3) -26.8	(1) 6.5 (2) Inf Dil (3) 8.08	(1) [149] (2) [101] (3) [16]	4.857	[101]	[3], [37]
Iodide	-70.2	6.5	[149]	n/a	n/a	[64], [80], [84], [90], [116], [139], [142], [154]
Lactate	-29.2	6.5	[149]	3.86	[149]	[36], [51], [68-69], [111-112], [119-120], [139], [148]
Lactose	(1) -6.94 (2) -9.5 (3) -4.2	(1) 12.2 (2) 12.1 (3) 12.1	(1) [108] (2) [155] (3) [152]	11.98	[108]	[133], [151]
Lactulose	(1) -8.16, (2) -5.4	(1) 12.2 (2) 12.1	(1) [108] (2) [152]	n/a	n/a	[151]
Laurate	-9	8	[28]	n/a	n/a	n/a
Leucine	-23	11	[6]	9.74	[6]	[98], [141]

See p. 16 for notes on the organisation of this Table. See p. 67 for references.

Table 1.8 (Continued) Analyte Data

Analyte	Mobility			*pKa*		Cross Ref.
	$10^{-9}m^2V^{-1}s^{-1}$	pH	Ref.		Ref.	
d-lyxose	(1) -10.03 (2) -13.9 (2) -6.4	(1) 12.2 (2) 12.1 (3) 12.1	(1) [108] (2) [155] (3) [152]	12.11	[108]	[151]
Malate	-45.8	6.5	[149]	(1) 3.4 (2) 5.05	[149]	[36], [40], [68], [109], [117], [119-120], [130]
Malonate	-61.1	8.08	[16]	n/a	n/a	[16], [51-52], [57], [111], [130], [132], [148]
Maltose	(1) -7.90 (2) -11.0 (3) -4.8	(1) 12.2 (2) 12.1 (3) 12.1	(1) [108] (2) [155] (3) [162]	11.94	[108]	[105], [133], [151-152]
Maltotriose	-4.3	12.1	[152]	n/a	n/a	[151]
d-mannitol	-4.2	12.1	[155]	n/a	n/a	n/a
d-mannonic acid	-25.7	12.1	[152]	n/a	n/a	[151]
d-mannose	(1) -10.25 (2) -12.8 (3) -7.5	(1) 12.2 (2) 12.1 (3) 12.1	(1) [108] (2) [155] (3) [152]	12.08	[108]	[151], [163]
d-mannuronic acid	-29.3	12.1	[152]	n/a	n/a	[151]
Meleziose	-4.2	12.1	[155]	n/a	n/a	n/a
Melibiose	(1) -7.12 (2) -7.7 (3) -4.6	(1) 12.2 (2) 12.1 (3) 12.1	(1) [108] (2) [155] (3) [152]	n/a	n/a	[151]
Methanesulfonate	-44.7	9.2	[136]	n/a	n/a	[41], [69], [129], [134]
(N-morpholino)-ethanesulfonic acid	-28	Inf Dil	[12]	6.095	[12]	[3]
Myristate	-8.4	8	[28]	n/a	n/a	n/a

See p. 16 for notes on the organisation of this Table. See p. 67 for references.

Table 1.8 (Continued) Analyte Data

Analyte	Mobility			*pKa*		Cross Ref.
	$10^{-9}m^2V^{-1}s^{-1}$	pH	Ref.		Ref.	
Nitrate	(1) -74.3 (2) -64.3	(1) Inf Dil (2) 6.5	(1) [14] (2) [149]	-1.34	[149]	[36], [38], [39-42], [46], [52], [55], [60-61], [63-66], [69-73], [76-77], [79-89], [91-97], [99], [116], [119-122], [127], [130], [132], [134], [138-139], [142], [154], [161]
Nitrilotriacetic acid	-52	6.5	[75]	n/a	n/a	n/a
Nonanesulfonate	-24.1	9.2	[136]	n/a	n/a	[69]
Octanesulfonate	-25.3	9.2	[136]	n/a	n/a	[41], [69], [146]
Octanoate	-20.7	6.5	[149]	n/a	n/a	n/a
Oleate	-7.7	8	[28]	n/a	n/a	[79]
Oxalate	-58.8	6.5	[149]	(1) 1.27 (2) 4.27	[149]	[36], [38], [40], [55-59], [68-70], [72], [99], [109], [111], [116], [119-120], [122], [130], [132], [134]
Palatinose	-5.6	12.1	[151]	n/a	n/a	n/a
Palmitate	-7.9	8	[28]	n/a	n/a	n/a
Pentanesulfonate	-29.7	9.2	[136]	n/a	n/a	[41], [60], [63], [69], [96], [146]

See p. 16 for notes on the organisation of this Table. See p. 67 for references.

Table 1.8 (Continued) Analyte Data

Analyte	Mobility			*pKa*		Cross Ref.
	$10^{-9}m^2V^{-1}s^{-1}$	pH	Ref.		Ref.	
Phosphate	-55	9	[75]	(1) 2.12 (2) 7.20 (3) 12.36	[149]	[2], [23], [36], [39-42] [51], [55], [57], [60-61], [63], [66], [69-72], [76], [79], [83-88], [90], [94], [115], [120-121], [127], [130], [132], [142], [161]
Proline	-16.8	11	[6]	10.64	[164]	[98], [141]
Propionate	-33.4	8.08	[16]	n/a	n/a	[3], [59-60], [63], [65], [69-70], [79], [128], [132], [148]
Propionate	-33.4	8.08	[16]	n/a	n/a	[37-38]
Pyrophosphate	-78	9	[75]	n/a	n/a	[23], [115], [118], [161]
Pyruvate	-33.4	6.5	[149]	2.49	[149]	[36], [45], [111], [113], [132], [139]
Raffinose	(1) -2.36 (2) -6.0 (3) -1.3	(1) 12.2 (2) 12.1 (3) 12.1	(1) [108] (2) [155] (3) [152]	12.74	[108]	[133], [151]
l-rhamnose	(1) -9.37 (2) -6.6	(1) 12.2 (2) 12.1	(1) [108] (2) [152]	n/a	n/a	[151]
d-ribonic acid	-28.1	12.1	[152],	n/a	n/a	[151]
d-ribose	(1) -10.65 (2) -13.9 (3) -7.4	(1) 12.2 (2) 12.1 (3) 12.1	(1) [108] (2) [155] (3) [152]	12.21	[108]	[151]
Saccharose	-1.4	12.1	[152]	n/a	n/a	[151]

See p. 16 for notes on the organisation of this Table. See p. 67 for references.

Table 1.8 (Continued) Analyte Data

Analyte	Mobility			*pKa*		Cross Ref.
	$10^{-9}m^2V^{-1}s^{-1}$	pH	Ref.		Ref.	
Serine	-29.5	11	[6]	9.26	[6]	[98], [141]
d-sorbitol	-4.2	12.1	[155]	n/a	n/a	n/a
d-sorbose	-12.5	12.1	[155]	n/a	n/a	n/a
l-sorbose	(1) -10.42 (2) -6.5	(1) 12.1 (2) 12.1	(1) [108], (2) [152],	11.5	[108]	[151]
Stachyose	-4.2	12.1	[155]	n/a	n/a	n/a
Stearate	-7.5	8	[28]	n/a	n/a	n/a
Succinate	-44.4	6.5	[149]	(1) 4.21 (2) 5.64	[149]	[36], [57], [69], [99], [109], [111], [117], [119-120], [130]
Sucrose	(1) -2.41 (2) -4.2	(1) 12.2 (2) 12.1	(1) [108] (2) [155]	12.51	[108]	[105], [133], [155]
Sulfate	(1) -70.8 (2) 83	(1) 8.0 (2) Inf Dil	(1) [29] (2) [77]	(1) -3 (2) 1.92	[149]	[2], [35-36], [38-42], [46], [52], [55-57], [59-61], [63-66], [69-73], [76], [79-89], [91] [93-97], [116], [119-122], [127], [130], [132], [134], [138], [154], [161]
Sulfide	-63.6	8	[29]	n/a	n/a	n/a
Tartrate	(1) -46.8 (2) -56.3	(1) 6.5 (2) 8.08	(1) [149] (2) [16]	(1) 2.98 (2) 4.34	[68]	[40], [57], [68], [109], [111], [117], [119-120], [130]
Tetrathionate	-65.3	8	[29]	n/a	n/a	n/a

See p. 16 for notes on the organisation of this Table. See p. 67 for references.

Table 1.8 (Continued) Analyte Data

Analyte	Mobility			*pKa*		Cross Ref.
	$10^{-9}m^2V^{-1}s^{-1}$	pH	Ref.		Ref.	
Thiosulfate	-77.3	8	[29]	n/a	n/a	[39-41], [59], [64], [69], [79], [88], [95], [116]
Thymidene 5'-diiphosphate	-37.34	3.5	[140]	n/a	n/a	[142]
Thymidene 5'-monophosphate	-19.13	3.5	[140]	n/a	n/a	[142]
Tripolyphosphate	-65	6.5	[75]	n/a	n/a	[23], [115]
Turanose	-9.52	12.2	[108]	n/a	n/a	[151]
Valerate	-24.5	6.5	[149]	n/a	n/a	[60], [63], [65]
Valine	-24.6	11	[6]	9.74	[6]	[98]
d-xylose	(1) -10.06 (2) -12.5 (3) -6.3	(1) 12.2 (2) 12.1 (3) 12.1	(1) [108] (2) [155] (3) [152]	12.29	[108]	[133], [151]

See p. 16 for notes on the organisation of this Table. See p. 67 for references.

1.12 References

[1] S. Hjerten, K. Elenbring, F. Kilar, J. Liao, A. J. C. Chen, C. J. Siebert and M. Zhu, *J. Chromatogr.*, 21 (1987) 47.

[2] S. M. Cousins, P. R. Haddad and W. Buchberger, *J. Chromatogr. A*, 671 (1994) 397.

[3] M. T. Ackermans, F. M. Everaerts and J. L. Beckers, *J. Chromatogr.*, 549 (1991) 345.

[4] R. Kuhn and S. Hoffstetter-Kuhn, Capillary Electrophoresis: Principles and Practice, Springer Laboratory, (1993).

[5] T. Rabilloud, *Electrophoresis*, 15 (1994) 278.

[6] G. J. M. Bruin, A. C. Van Asten, X. Xu and H. Poppe, *J. Chromatogr.*, 608 (1992) 97.

[7] F. E. P. MIkkers, F. M. Everaerts and T. P. E. M. Verheggen, *J. Chromatogr.*, 169 (1979) 1.

[8] H. Poppe, *Anal. Chem.*, 64 (1992) 1908.

[9] M. W. F. Nielen, *J. Chromatogr.*, 588 (1991) 321.

[10] W. Buchberger, S. M. Cousins and P. R. Haddad, *TrAC,*, 13 (1994) 313.

[11] X. Xu, W. T. Kok and H. Poppe, *J . Chromatogr.*, 742 (1996) 211.

[12] J. L. Beckers, *J. Chromatogr. A*, 693 (1995) 347.

[13] J. L. Beckers, *J. Chromatogr.*, 741 (1996).

[14] J. L. Beckers, *J. Chromatogr.*, 764 (1997) 111.

[15] F. E. P. Mikkers, F. M. Everaerts and T. P. E. M. Verheggen, *J. Chromatogr.*, 169 (1979) 11.

[16] T. Wang and R. A. Hartwick, *J. Chromatogr.*, 589 (1992) 307.

[17] J. L. Beckers, *J. Chromatogr. A*, 679 (1994) 153.

[18] M. Macka, P. R. Haddad, P. Gebauer and P. Bocek, *Electrophoresis*, 18 (1997) 1998.

[19] C. O. Thompson, V. C. Trenerry and B. Kemmery, *J. Chromatogr., A*, 704 (1995) 203.

[20] A. Henshall, M. P. Harrold and J. M. Y. Tso, *J. Chromatogr.*, 608 (1992) 413.

[21] B. A. P. Buscher, U. R. Tjaden, H. Irth, E. M. Andersson and J. van der Greef, *J. Chromatogr., A*, 718 (1995) 413.

[22] L. K. Goebel, H. M. McNair, H. T. Rasmussen and B. P. McPherson, *J. Microcolumn Sep.*, 5 (1993) 47.

[23] S. A. Shamsi and N. D. Danielson, *Anal. Chem.*, 67 (1995) 1845.

[24] S. A. Shamsi and N. D. Danielson, *Anal. Chem.,* 67 (1995) 4210.

[25] G. A. Pianetti, M. Taverna, A. Baillet, G. Mahuzier and D. Baylocq Ferrier, *J. Chromatogr.,* 630 (1993) 371.

[26] R. W. Hepler and C. C. Yu Ip, *J. Chromatogr. A,* 680 (1994) 201.

[27] S. A. Oehrle and P. C. Bossle, *J. Chromatogr. A,* 692 (1995) 247.

[28] R. Neubert, K. Raith and J. Schiewe, *Pharmazie,* 52 (1997) 212.

[29] S. Motellier, K. Gurdale and H. Pitsch, *J. Chromatogr.,* 770 (1997) 311.

[30] K. L. Larsen, F. Mathiesen and W. Zimmermann, *Carbohydrate Research,* 298 (1997) 59.

[31] R. Roldan Assad and P. Gareil, *J. Chromatogr., A,* 708 (1995) 339.

[32] A. Nardi, S. Fanali and F. Foret, *Electrophoresis,* 11 (1990) 774.

[33] E. V. Dose and G. A. Guiochon, *Anal. Chem.,* 63 (1991) 1063.

[34] C. Vogt, J. Vogt and H. Wittrisch, *J. Chromatogr., A,* 727 (1996) 301.

[35] C. Vogt and G. Werner, *J. Chromatogr. A,* 686 (1994) 325.

[36] A. Bazzanella, H. Lochmann, A. Mainka and K. Bachmann, *Chromatographia,* 45 (1997) 59.

[37] T. Wang and R. A. Hartwick, *J. Chromatogr.,* 607 (1992) 119.

[38] E. Dabek Zlotorzynska and J. F. Dlouhy, *J. Chromatogr. A,* 671 (1994) 389.

[39] M. M. Rhemrev Boom, *J. Chromatogr. A,* 680 (1994) 675.

[40] T. Ehmann, K. Bachmann, L. Fabry, H. Rufer, S. Pahlke and L. Kotz, *Chromatographia,* 45 (1997) 301.

[41] M. P. Harrold, M. J. Wojtusik, J. Riviello and P. Henson, *J. Chromatogr.,* 640 (1993) 463.

[42] M. J. Wojtusik and M. P. Harrold, *J. Chromatogr. A,* 671 (1994) 411.

[43] M. Macka and P. Haddad, *Electrophoresis,* (1998).

[44] M. Albin, P. D. Grossman and S. E. Moring, *Anal. Chem.,* 65 (1993) 489a.

[45] Y. J. Xue and E. S. Yeung, *Anal. Chem.,* 65 (1993) 2923.

[46] Y. F. Ma and R. L. Zhang, *J. Chromatogr.,* 625 (1992) 341.

[47] E. S. Yeung and W. G. Kuhr, *Anal. Chem.,* 63 (1991) 280a.

[48] A. Weston, P. R. Brown, P. Jandik, A. L. Heckenberg and W. R. Jones, *J. Chromatogr.,* 608 (1992) 395.

[49] F. Steiner, W. Beck and H. Engelhardt, *J. Chromatogr., A,* 738 (1996) 11.

[50] P. Jandik and W. R. Jones, *J. Chromatogr.,* 546 (1991) 431.

[51] F. Foret, S. Fanali, L. Ossicini and P. Bocek, *J. Chromatogr.,* 470 (1989) 299.

[52] Z. Mala, R. Vespalec and P. Bocek, *Electrophoresis,* 15 (1994) 1526.

[53] W. Beck and H. Engelhardt, *Chromatographia,* 33 (1992) 313.

[54] J. Aupiais, *Chromatographia,* 44 (1997) 303.

[55] B. J. Wildman, P. E. Jackson, W. R. Jones and P. G. Alden, *J. Chromatogr.,* 546 (1991) 459.

[56] S. C. Grocott, L. P. Jefferies, T. Bowser, J. Carnevale and P. E. Jackson, *J. Chromatogr.,* 602 (1992) 257.

[57] P. R. Haddad, A. H. Harakuwe and W. W. Buchberger, *J. Chromatogr., A,* 706 (1995) 571.

[58] A. H. Harakuwe, P. R. Haddad and P. E. Jackson, *J. Chromatogr., A,* 739 (1996) 399.

[59] D. R. Salomon and J. Romano, *J. Chromatogr.,* 602 (1992) 219.

[60] W. R. Jones and P. Jandik, *J. Chromatogr.,* 608 (1992) 385.

[61] J. P. Romano and J. Krol, *J. Chromatogr.,* 640 (1993) 403.

[62] W. R. Jones and P. Jandik, *Am. Lab.,* 22 (1990) 51.

[63] W. R. Jones and P. Jandik, *J. Chromatogr.,* 546 (1991) 445.

[64] W. Buchberger and P. R. Haddad, *J. Chromatogr.,* 608 (1992) 59.

[65] N. J. Benz and J. S. Fritz, *J. Chromatogr. A,* 671 (1994) 437.

[66] A. H. Harakuwe, P. R. Haddad and W. Buchberger, *J. Chromatogr. A,* 685 (1994) 161.

[67] C. A. Lucy and T. L. McDonald, *Anal. Chem.,* 67 (1995) 1074.

[68] C. H. Wu, Y. S. Lo, Y. H. Lee and T. I. Lin, *J. Chromatogr., A,* 716 (1995) 291.

[69] J. Romano, P. Jandik, W. R. Jones and P. E. Jackson, *J. Chromatogr.,* 546 (1991) 411.

[70] G. Bondoux, P. Jandik and W. R. Jones, *J. Chromatogr.,* 602 (1992) 79.

[71] S. A. Oehrle, *J. Chromatogr., A,* 733 (1996) 101.

[72] S. A. Oehrle, *J. Chromatogr., A,* 745 (1996) 81.

[73] K. D. Altria, D. M. Goodall and M. M. Rogan, *Chromatographia,* 38 (1994) 637.

[74] J. C. Transfiguracion, C. Dolman, D. H. Eidelman and D. K. Lloyd, *Anal. Chem.,* 67 (1995) 2937.

[75] T. L. Wang and S. F. Y. Li, *J. Chromatogr., A,* 723 (1996) 197.

[76] R. Saari Nordhaus and J. M. Anderson, Jr., *J. Chromatogr., A,* 706 (1995) 563.

[77] J. Boden, K. Baechmann, L. Kotz, L. Fabry and S. Pahlke, *J. Chromatogr., A,* 696 (1995) 321.

[78] L. Lin, J. Wang and J. Caruso, *J. Chromatogr. Sci.,* 33 (1995) 177.

[79] M. Jimidar, C. Harmann, N. Cousement and D. L. Massart, *J. Chromatogr., A,* 706 (1995) 479.

[80] M. Jimidar, B. Bourguignon and D. L. Massart, *Anal. Chim. Acta,* 310 (1995) 27.

[81] K. Bachmann, I. Haag, T. Prokop, A. Roeder and P. Wagner, *J. Chromatogr.,* 643 (1993) 181.

[82] C. Stathakis and R. M. Cassidy, *Anal. Chem.,* 66 (1994) 2110.

[83] M. T. Galceran, L. Puignou and M. Diez, *J. Chromatogr., A,* 732 (1996) 167.

[84] A. H. Harakuwe and P. R. Haddad, *J. Chromatogr., A,* 734 (1996) 416.

[85] M. E. Swartz, *J. Chromatogr.,* 640 (1993) 441.

[86] P. Morin, C. Francois and M. Dreux, *Analusis,* 22 (1994) 178.

[87] C. Francois, P. Morin and M. Dreux, *J. High Resol. Chromatogr. 19 (1996) 5.*

[88] K. Li and S. F. Y. Li, *J. Liq. Chromatogr.,* 17 (1994) 3889.

[89] S. A. Oehrle, *J. Chromatogr. A,* 671 (1994) 383.

[90] M. Jimidar, Q. Yang, J. Smeyers Verbeke and D. L. Massart, *TrAC.,* 15 (1996) 91.

[91] G. Y. Jung, T. H. Kim and H. B. Lim, *Anal. Sci.,* 12 (1996) 367.

[92] E. S. Kwak and F. A. Gomez, *Chromatographia,* 43 (1996) 659.

[93] J. D. Lamb, T. L. Huxford and K. B. Czirr, *J. Chromatogr., A,* 739 (1996) 373.

[94] P. Wang, S. F. Y. Li and H. K. Lee, *J. Chromatogr.,* 765 (1997) 353.

[95] G. W. Tindall and R. L. Perry, *J. Chromatogr., A,* 696 (1995) 349.

[96] W. R. Jones, *J. Chromatogr.,* 640 (1993) 387.

[97] W. Tong and E. S. Yeung, *J. Chromatogr., A,* 718 (1995) 177.

[98] Y. H. Lee and T. I. Lin, *J. Chromatogr. A,* 680 (1994) 287.

[99] B. Tenberken, P. Ebert, M. Hartmann, M. Kibler, A. Mainka, T. Prokop, A. Roder and K. Bachmann, *J. Chromatogr.,* 745 (1996) 209.

[100] R. J. Tait, D. J. Skanchy, D. P. Thompson, N. C. Chetwyn, D. A. Dunshee, R. A. Rajewski, V. J. Stella and J. F. Stobaugh, *J. Pharm. Biomed. Anal.,* 10 (1992) 615.

[101] J. L. Beckers, *J. Chromatogr., A,* 696 (1995) 285.

[102] E. A. Luna, E. R. N. Bornancini, D. O. Thompson, R. A. Rajewski and V. J. Stella, *Carbohydrate Research,* 299 (1997) 103.

[103] J. M. Gibbons and S. H. Hoke, *J. High Resolut. Chromatogr.,* 17 (1994) 665.

[104] K. Baechmann, I. Haumann and T. Groh, *Fresenius' J. Anal. Chem.,* 343 (1992) 901.

[105] X. Xu, W. T. Kok and H. Poppe, *J. Chromatogr., A,* 716 (1995) 231.

[106] G. Gutnikov, W. Beck and H. Engelhardt, *J. Microcolumn Sep,* 6 (1994) 565.

[107] L. Zhou and A. Dovletoglou, *Journal of Chromatography,* 763 (1997) 279.

[108] Y. H. Lee and T. I. Lin, *J. Chromatogr., B,* 681 (1996) 87.

[109] S. P. D. Lalljie, J. Vindevogel and P. Sandra, *J. Chromatogr. A,* 652 (1993) 563.

[110] F. Y. Guan, H. F. Wu and Y. Luo, *J. Chromatogr., A,* 719 (1996) 421.

[111] H. Chen, Y. Xu, F. Van Lente and M. P. C. Ip, *J. Chromatogr., B,* 679 (1996) 49.

[112] D. Lagoutte, G. Lombard, S. Nisseron, M. P. Papet and Y. Saint Jalm, *J. Chromatogr. A,* 684 (1994) 251.

[113] D. Volgger, A. J. Zemann, G. K. Bonn and M. J. Antal, *J. Chromatogr.,* 758 (1997) 263.

[114] M. G. Cikalo, D. M. Goodall and W. Matthews, *J. Chromatogr.,* 745 (1996) 189.

[115] F. S. Stover and S. S. Keffer, *J. Chromatogr. A,* 657 (1993) 450.

[116] H. Salimi Moosavi and R. M. Cassidy, *Anal. Chem.,* 67 (1995) 1067.

[117] K. D. Altria, K. H. Assi, S. M. Bryant and B. J. Clark, *Chromatographia,* 44 (1997) 367.

[118] P. E. Andersson, W. D. Pfeffer and L. G. Blomberg, *J. Chromatogr., A,* 699 (1995) 323.

[119] L. Kelly and R. J. Nelson, *J. Liq. Chromatogr.,* 16 (1993) 2103.

[120] M. Arellano, J. Andrianary, F. Dedieu, F. Couderc and P. Puig, *J. Chromatogr.,* 765 (1997) 321.

[121] H. Burt, D. M. Lewis and K. N. Tapley, *J. Chromatogr., A,* 739 (1996) 367.

[122] E. Dabek Zlotorzynska, J. F. Dlouhy, N. Houle, M. Piechowski and S. Ritchie, *J. Chromatogr., A,* 706 (1995) 469.

[123] S. Chen and D. J. Pietrzyk, *Anal. Chem.,* 65 (1993) 2770.

[124] Y. H. Lee and T. I. Lin, *Electrophoresis, Feb,* 17 (1996) 333.

[125] J. B. L. Damm and G. T. Overklift, *J. Chromatogr. A,* 678 (1994) 151.

[126] W. Buchberger and P. R. Haddad, *J. Chromatogr. A,* 687 (1994) 343.

[127] M. Van Holderbeke, H. Vanhoe, L. Moens and R. Dams, *Biomed. Chromatogr.,* 9 (1995) 281.

[128] G. W. Tindall, D. R. Wilder and R. L. Perry, *J. Chromatogr.,* 641 (1993) 163.

[129] E. Dabek Zlotorzynska and J. F. Dlouhy, *J. Chromatogr. A,* 685 (1994) 145.

[130] S. A. Shamsi and N. D. Danielson, *Anal. Chem.,* 66 (1994) 3757.

[131] B. A. P. Buscher, H. Irth, E. Andersson, U. R. Tjaden and J. van der Greef, *J. Chromatogr. A,* 678 (1994) 149.

[132] H. Siren, A. Maattanen and M. L. Riekkola, *J. Chromatogr.,* 767 (1997) 293.

[133] T. W. Garner and E. S. Yeung, *J. Chromatogr.,* 515 (1990) 639.

[134] T. Groh and K. Baechmann, *Electrophoresis,* 13 (1992) 458.

[135] I. H. Grant and W. Steuer, *J. Microcolumn Sep,* 2 (1990) 74.

[136] A. M. Desbene, C. J. Morin, N. L. Mofaddel and R. S. Goulf, *J. Chromatogr., A,* 716 (1995) 279.

[137] Y. C. Chao and C. W. Whang, *J. Chromatogr. A,* 663 (1994) 229.

[138] V. Marti, M. Aguilar and E. S. Yeung, *J. Chromatogr., A,* 709 (1995) 367.

[139] Q. Xue and E. S. Yeung, *J. Chromatogr. A,* 661 (1994) 287.

[140] W. G. Kuhr and E. S. Yeung, *Anal. Chem.,* 60 (1988) 2642.

[141] W. G. Kuhr and E. S. Yeung, *Anal. Chem.,* 60 (1988) 1832.

[142] L. Gross and E. S. Yeung, *J. Chromatogr.,* 480 (1989) 169.

[143] B. L. Hogan and E. S. Yeung, *J. Chromatogr. Sci.,* 28 (1990) 15.

[144] M. W. F. Nielen, *J. Chromatogr.,* 608 (1992) 85.

[145] D. F. Swaile, C. L. Copper, M. J. Sepaniak, D. E. Burton and L. L. Powell, *Talanta,* 41 (1994) 1499.

[146] S. A. Shamsi and N. D. Danielson, *J. Chromatogr., A,* 739 (1996) 405.

[147] W. Buchberger and K. Winna, *Mikrochim. Acta,* 122 (1996) 45.

[148] C. W. Klampfl and W. Buchberger, *TrAC,* 16 (1997) 221.

[149] T. Soga and G. A. Ross, *J. Chromatogr.,* 767 (1997) 223.

[150] A. Klockow, A. Paulus, V. Figueiredo, R. Amado and H. M. Widmer, *J. Chromatogr. A,* 680 (1994) 187.

[151] A. E. Vorndran, P. J. Oefner, H. Scherz and G. K. Bonn, *Chromatographia,* 33 (1992) 163.

[152] P. J. Oefner, A. E. Vorndran, E. Grill, C. Huber and G. K. Bonn, *Chromatographia,* 34 (1992) 308.

[153] W. H. Robins and B. W. Wright, *J. Chromatogr., A,* 680 (1994) 667.

[154] J. Boden, T. Ehmann, T. Groh, I. Haumann and K. Baechmann, *Fresenius' J. Anal. Chem,* 348 (1994) 572.

[155] B. Lu and D. Westerlund, *Electrophoresis,* 17 (1996) 325.

[156] J. Collet and P. Gareil, *J. Chromatogr., A,* 716 (1995) 115.

[157] K. D. Altria and J. S. Howells, *J. Chromatogr., A,* 696 (1995) 341.

[158] S. Conradi, C. Vogt, H. Wittrisch, G. Knobloch and G. Werner, *J. Chromatogr.,* 745 (1996) 103.

[159] J. Pospichal, P. Gebauer and P. Bocek, *Chem. Rev.,* 89 (1989) 419.

[160] C. L. Colyer, K. B. Oldham and A. V. Sokirko, *Anal. Chem.,* 67 (1995) 3234.

[161] J. Kerth and D. Jensen, *J. Chromatogr., A,* 706 (1995) 191.

[162] I. Recio and C. Olieman, *Electrophoresis,* 17 (1996) 1228.

[163] G. Li and D. C. Locke, *J. Chromatogr., A,* 734 (1996) 357.

[164] K. D. Altria, M. A. Kelly and B. J. Clark, *Chromatographia,* 43 (1996) 153.

2. Experimental

In this section the instrumentation, chemicals and procedures used throughout this work (unless specified later in a particular chapter) are given.

2.1 Instrumentation

2.1.1 CE Hardware

The CE instruments used were:

Applied Biosystems Model 270A-HT (Perkin-Elmer, San Jose, USA) interfaced to Turbochrom chromatography software (Perkin-Elmer).

Quanta 4000 (Waters, Milford, MA, USA) interfaced to a Maxima 820 data station (Waters).

2.1.2 Capillaries

All separations were performed using unmodified fused silica capillaries purchased from Polymicro Technologies Inc. (Phoenix, AZ, USA), 75 μm i.d. 0.60 m total length, 0.52 m to detector cell (Quanta 4000); or 0.70 m total length, 0.50 m to detector cell (270A-HT).

The outer polyimide coating of the capillary was burnt at the point of detection in a length of approx. 2 mm and cleaned with acetone.

2.2 Reagents

Unless otherwise specified all chemicals are of analytical grade.

Table 2.1 Chemicals used as Buffers

Buffer	*pKa*	Formula	Supplier
1,3-bis-[tris(hydroxymethyl)-methylamino]-propane (Bis Tris)	6.8	$C_{11}H_{26}N_2O_6$	Sigma, St Louis, MO
2-(cyclohexylamino)ethanesulfonic acid (CHES)	9.55	$C_8H_{17}NO_3S$	Sigma
d-glutamic acid	3.22 (p*I*)	$C_5H_9NO_4$	Sigma
diethanolamine (DEA)	9.2	$C_4H_{11}NO_2$	Fluka, Buchs, Switzerland
lysine	9.7 (p*I*)	$C_6H_{14}N_2O_2$	Sigma; Fluka; Aldrich, Milwaukee, WI
tris(hydroxymethyl)aminoethane (Tris)	8.1	$C_4H_{11}NO_3$	Sigma

Table 2.2 Chemicals used as Probes

Probe	Formula	Supplier
4-hydroxybenzenesulfonic acid (65% w/w in H_20)	$HOC_6H_4SO_3H$	Aldrich
4-hydroxybenzoic acid	$C_7H_6O_3$	Sigma
benzoic acid	$C_7H_6O_2$	Sigma
bromocresol green (BCG)- dye content 95%		Aldrich
chromium trioxide	CrO_3	Fluka
naphthalenedisulfonic acid	$C_{10}H_6(SO_3H)_2$	Aldrich
phthalic acid	$C_6H_4(CO_2H)_2$	Aldrich
potassium indigo-tetrasulfonate (ITS) - dye content 85%		Aldrich
p-toluenesulfonic acid	$CH_3C_6H_4SO_3H$	Aldrich
sodium chromate	Na_2Cr0_4	Aldrich

Probe	Formula	Supplier
sulfophthalic acid (% w/w in H20)	$H0_3SC_6H_3(CO_2H)_2$	
trimellitic acid	$C_6H_3(CO_2H)_3$	Aldrich

Table 2.3 Chemicals used as Analytes

Analyte	Formula	Supplier
butanesulfonate (sodium salt)	$CH_3(CH2)_3\ SO_3Na$	Aldrich
ethanesulfonate (sodium salt)	$CH_3CH_2SO_3Na$	Aldrich
heptanesulfonate (sodium salt)	$CH_3(CH_2)_6\ SO_3Na$	Aldrich
hexanesulfonate (sodium salt)	$CH_3(CH_2)_5\ SO_3Na$	Aldrich
hydrochloric acid	HCl	Ajax Chemicals, Sydney, Australia
mesityl oxide	$CH_3CCHCOCH_3$	Aldrich
methanesulfonate (sodium salt)	CH_2SO_3Na	Aldrich
octanesulfonate (sodium salt)	$CH_3(CH_2)_7\ SO_3Na$	Aldrich
pentanesulfonate (sodium salt)	$CH_3(CH_2)_4\ SO_3Na$	Aldrich
perchloric acid	$HClO_4$	Ajax Chemicals
propanesulfonate (sodium salt)	$CH_3(CH_2)_2\ SO_3Na$	Aldrich
propanoic acid	CH_3CH_2COOH	Aldrich
sodium bromide	NaBr	Ajax Chemicals
sodium carbonate	$NaCO_3$	Ajax Chemicals
sodium cyanate	NaOCN	Aldrich
sodium dihydrogen phosphate	NaH_2PO_4	Ajax Chemicals
sodium formate	$NaCO_2H$	Ajax Chemicals

Analyte	Formula	Supplier
sodium iodide	NaI	Ajax Chemicals
sodium nitrate	$NaNO_3$	Ajax Chemicals
sodium nitrite	$NaNO_2$	Ajax Chemicals
sodium sulfate	Na_2SO_4	Ajax Chemicals
sodium thiocyanate	NaSCN	Aldrich
sulfuric acid	H_2SO_4	Ajax Chemicals

2.3 Procedures

2.3.1 Electrolyte and Standard Preparation

All carrier BGEs and standard solutions of analytes were prepared in water treated with a Millipore (Bedford, MA. USA) Milli-Q water purification apparatus. BGEs were degassed using vacuum sonication and were filtered through a 0.45 μm syringe filter (Activon, Thornleigh, Australia) prior to use.

2.3.2 Sample Injection

For the Quanta 4000 (Waters), injections were performed hydrostatically by elevating the sample at 10 cm usually between 5 and 20 secs. For the Model 270A-HT (Perkin Elmer) injections were performed under vacuum at 5" Hg usually for 0.6 to 1.2s.

2.3.3 Calculation of electrophoretic mobilities

Electrophoretic mobilities were calculated using equations in reference [1] using mesityl oxide as the neutral marker for direct UV and water for indirect UV measurements to determine the rate of electroosmotic flow (EOF). The migration time of each analyte was taken as the apex of the peak.

2.3.4 Baseline Noise Measurements

Baseline noise was measured as described in reference [2]. Using digital data on a selected linear piece of baseline, linear regression and then statistical analysis of the residuals were performed and finally the noise was determined as five times the calculated standard deviation of the data points at the selected baseline interval.

2.4 References

[1] R. Kuhn and S. Hoffstetter-Kuhn, Capillary Electrophoresis: Principles and Practice, Springer Laboratory, 1993, pp 11-22.

[2] M. Macka, L. Semenkova, J. Borak, V. Mikes and M. Popl, *J. Liq. Chromatogr.*, 16 (1993) 2359.

3. Determination and Prediction of Transfer Ratios for Anions

3.1 Introduction

The ability of the analyte to displace the probe can be measured using the transfer ratio (TR), which is defined as the number of moles of the probe displaced by one mole of sample ions. It is desirable to know the value of TR for any given system since higher values result in larger peak areas and thus improved sensitivity. The purpose of this work was to demonstrate the conditions under which TR values calculated using eqn. (1.2) discussed in the literature review (pg. 4) are in agreement with those determined from experiment, and to provide explanations for non-ideal behaviour. Further, it was envisaged that a more complete understanding of the factors which influence transfer ratios would lead to the design of electrolyte systems which give maximum transfer ratios and hence the greatest detection sensitivity.

3.2 Experimental

The general experimental is given in Chapter Two. Detailed conditions are included in each of the figure captions.

3.2.1 Experimental Determination of Transfer Ratios

Transfer ratios were determined using the following steps [1]. First, a calibration plot was constructed of corrected peak area *versus* molar concentration of the analyte using a suitable probe as the carrier electrolyte and indirect UV detection. The correction made to peak area was to account for the different migration velocities of the sample bands and was achieved by dividing the peak area of each analyte by its migration time. Second, a corrected calibration plot for the probe was constructed using a suitable UV-transparent electrolyte and the same detection wavelength employed for the analyte calibration curve. Finally, TR values were calculated for each analyte ion by determining the quotient of the slope of the analyte ion calibration plot and the slope of the probe calibration plot.

3.3 Results and Discussion

3.3.1 Modification of the Detector

Since the TR is a quotient of the slopes of two calibration curves determined at different background absorbances, it is imperative that the response of the detector is linear over the absorbance range within which measurements are made or significant error will result. Foret *et al.* [3] states that a UV detector in CZE is linear up to *ca.* 0.1 AU, with the upper limit being determined mainly by the shape of the capillary detection cell. In our case, measurements for the Waters Quanta 4000 instrument made using a zinc lamp at 214 nm exhibited poor linearity up to 0.1 A.U. This was caused by stray light and poor alignment of the source. The lamp in the detector was designed with a broad window to maximise the light intensity, however this arrangement allowed an unacceptable level of stray light.

The detector optics were modified to reduce the stray light by wrapping aluminium foil around the lamp and two slits approximately 4 mm apart and 1mm in width were cut into it. This arrangement prevented light straying from the reference side of the detector to the sample side. The lamp was then moved approximately 1 cm away from the capillary and aligned so that the beam shone directly through the capillary. After these modifications the stray light was reduced to less than 4% and detector linearity was maintained up to *ca.* 0.4 AU. However, the lamp energy was diminished by a factor of five, which resulted in an increase in noise and therefore reduced sensitivity. Nevertheless, the improved linearity of the detector was essential to the determination of TR values so the modified optics were used for all experiments. When detection was performed at 254 nm, a deuterium lamp was modified in the same manner as described above.

3.3.2 Model Electrolyte-Analyte Systems

Several constraints must be considered in designing a simple electrolyte-analyte system that might show agreement between experimentally determined and calculated TR values. First, the background electrolyte must consist of two

components only: the probe anion and its corresponding counter-cation. Second, the analyte anion must have the same counter-cation as the background electrolyte. Third, the background electrolyte should have sufficient buffering capacity to prevent variations in EOF due to changes in electrolyte pH [4]. However, addition of buffers adversely affects the sensitivity of detection since displacement of the buffer anion (rather than the probe anion) by the analyte anion can occur [5]. Finally, the calibration plots necessary to calculate TR values must be performed within the linear range of the detector.

Initial experiments were conducted using two systems that satisfied these constraints. The first used chloride, sulfate, cyanate and formate as the analytes, with indirect detection at 214 nm in an electrolyte comprising 2.1 m*M* nitric acid, titrated with Bis-Tris to pH 6.9 (which is the pKa value of the buffer). This provided a buffered electrolyte that consisted of two components, namely the nitrate ion as the probe anion and the protonated Bis-Tris as the counter-cation. The power supply was set up so that the detector was at the anodic end of the capillary. A counter-EOF separation was used in which the anionic analytes migrated in a direction opposite to the EOF, but with sufficient effective velocity to reach the detector cell. Figure *3.1* shows the separation obtained under these conditions. The EOF velocity was determined for subsequent mobility calculations by switching the polarity of the power supply and injecting water. Calibration curves were constructed using sodium salts of the analytes dissolved in the electrolyte to negate the effect of the sodium ions. That is, calculation of the TR value was performed by considering the counter-cation of the analyte to be the Bis-Tris cation.

The second electrolyte-analyte system used a series of aliphatic sulfonates (C_3-C_8) as analytes and an electrolyte comprising 2.0 m*M* chromic acid titrated to pH 8 with Tris buffer, with detection at 254 nm using a deuterium lamp modified as described earlier. Again, this provided a buffered electrolyte containing only two components, namely chromate (as the probe anion) and protonated Tris as the counter-cation. The aliphatic sulfonates have low electrophoretic mobilities that are less than the EOF, so the power supply was set up with the detector at the cathodic end of the capillary.

Under these conditions, a counter-EOF mode was employed in which the effective velocity of the analytes was towards the detector. Figure *3.2* shows the separation obtained.

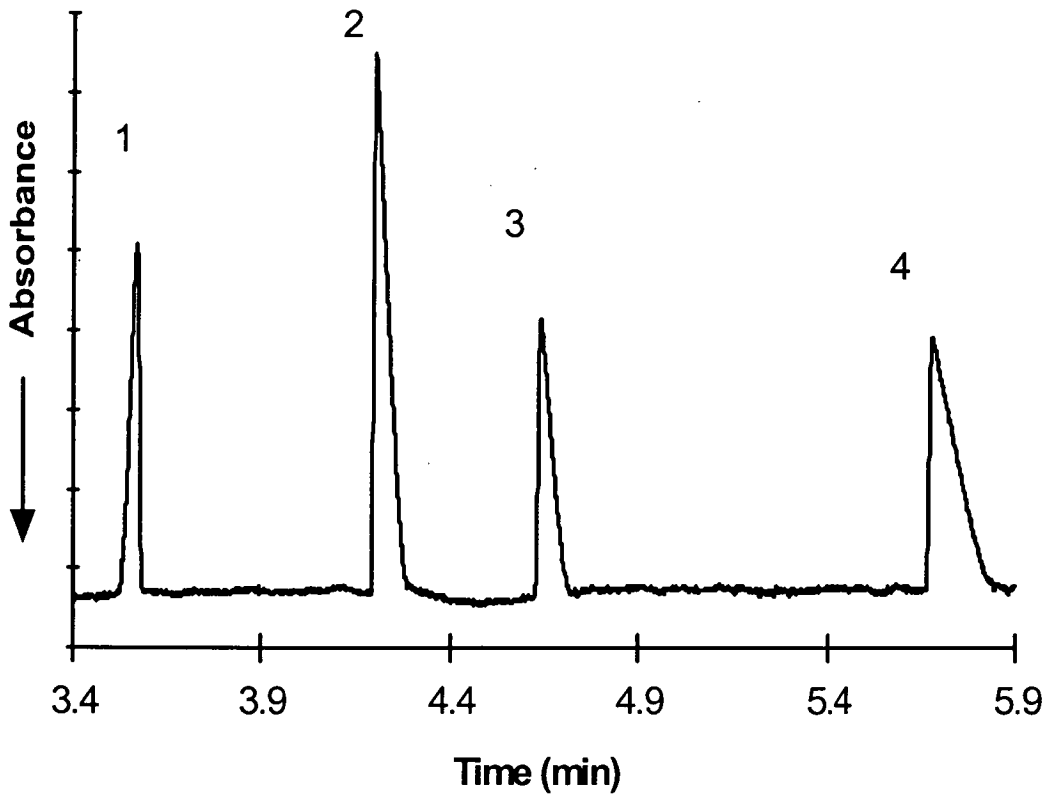


Figure 3.1 *Separation of chloride, sulfate, cyanate and formate using 2.1mM HNO_3 buffered to pH 6.8 with Bis-Tris. Conditions: separation voltage -25kV, indirect detection at 214 nm, hydrostatic injection at 10 cm for 10 sec, temperature 25°C, background absorbance 0.109 a.u., sample 0.2 mM of each anion. Key: 1 = chloride, 2 = sulfate, 3 = cyanate, 4 = formate.*

3.3.3 Effects of Ionic Strength of Standards

As described in the experimental section, the determination of TR involved preparation of calibration plots for both the probe (in the direct mode) and the analyte (in the indirect mode). When the probe calibration was performed for the first electrolyte system, an electrolyte comprising perchloric acid titrated to pH 6.9 with Bis-Tris was used and sodium nitrate was the injected sample. This provided a UV-transparent electrolyte enabling direct detection of nitrate at 214 nm. However, under these conditions it was found that the slope of the calibration plot for nitrate was dependent on the ionic strength of the electrolyte. A more concentrated electrolyte gave a higher slope for the calibration plot. This problem was overcome by dissolution of the analyte standards in electrolyte rather than water.

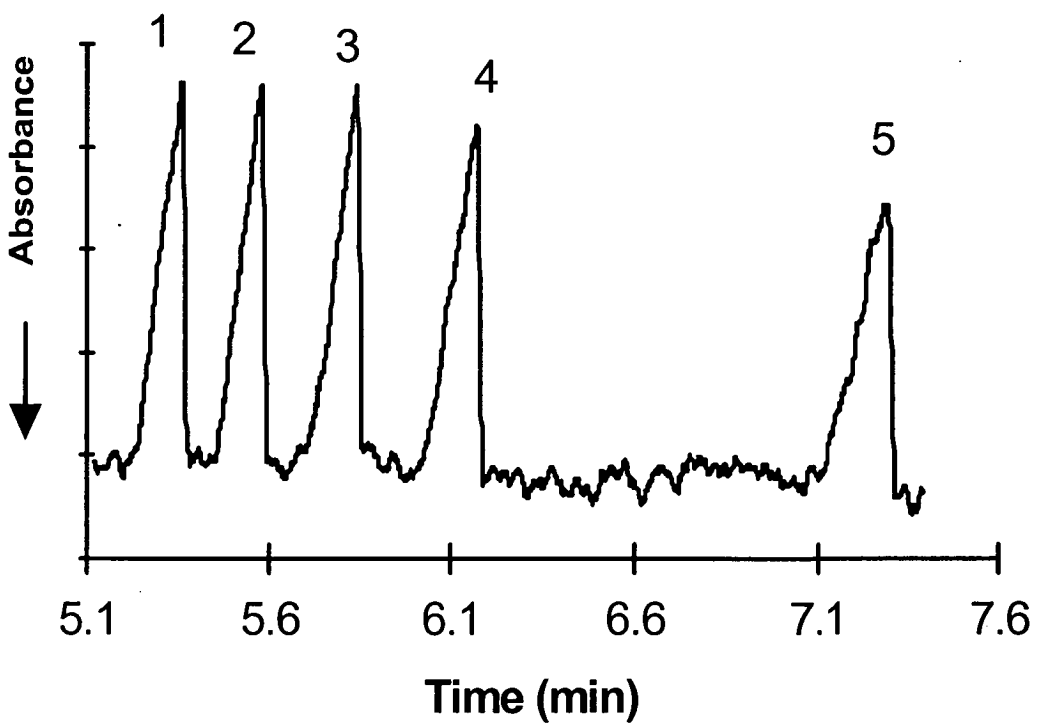


Figure 3.2 *Separation of aliphatic sulfonates using 2.0 mM chromic acid buffered to pH 8.0 with Tris. Conditions: separation voltage 25kV, indirect detection at 254 nm, hydrostatic injection at 10 cm for 10 sec, temperature 25°C, sample 0.3 mM of each anion. Key: 1 = octanesulfonate, 2 = heptanesulfonate, 3 = hexanesulfonate, 4 = pentanesulfonate, 5 = propanesulfonate.*

The same effect occurred also when constructing the calibration plots for the analytes in the indirect mode using nitrate as the probe. Table 3.1 illustrates the magnitude of the variation in slope when the analytes were dissolved in water or in the electrolyte. Doubling the ionic strength of the electrolyte produced increases in slope of up to 21.6% compared to the standards dissolved in water, whereas agreement was within 6% for the standards dissolved in the electrolyte and the variation could be attributed to random errors. For all subsequent TR determinations the standards were dissolved in the electrolyte.

3.3.4 Experimental Transfer Ratios for Model Electrolyte-Analyte Systems

Transfer ratios for the analytes in both model electrolyte systems were determined experimentally and theoretical values were calculated using eqn. (1.2). To permit the calculation, it was necessary to measure the mobilities of the Bis-Tris and Tris cations. These mobilities were determined using 10 m*M* dihydrogen phosphate adjusted to pH 6.8 (for Bis-Tris) or pH 8.0 (for Tris) with NaOH as the electrolyte, with detection at 185 nm.

The experimentally determined values of TR for the analytes are shown in Table 3.2, together with the calculated values. For both electrolytes agreement between experiment and theory is good, unlike earlier results [1] obtained using electrolyte systems and detectors that had not been optimised for the determination of TR values. In the case of the nitrate electrolyte the mobility of the probe is similar to that of the analytes so the experimental TR values show that displacement of the probe by the analyte occurs almost on an equivalent-per-equivalent basis. This displacement ratio is exceeded when the mobility of the probe is greater than that of the analyte, for example in the case of formate, and the reverse situation is also true using chloride as an example. As a further observation, the migration times of the analytes were very reproducible (relative standard deviations around 0.1%), which demonstrates the desirability of buffered electrolytes.

Table 3.1 **Slopes of calibration curves for different concentration of background electrolyte using standards dissolved in water or the electrolyte**

Analyte	Electrolyte: 4.2 m*M* HNO_3, 3.6 m*M* Bis-Tris, pH 6.8	Electrolyte: 2.1 m*M* HNO_3, 1.8 m*M* Bis-Tris, pH 6.8
Dissolved in water	Slope ± S.D.	Slope ± S.D.
Chloride	20900 ± 400	18900 ± 500
Sulfate	42600 ± 600	39000 ± 400
Cyanate	22400 ± 200	21500 ± 200
Formate	26100 ± 700	21400 ± 600
Dissolved in electrolyte		
Chloride	21000 ± 700	20700 ± 100
Sulfate	39300 ± 500	40400 ± 300
Cyanate	19200 ± 400	20300 ± 400
Formate	24300 ± 1100	23000 ± 1500

Conditions: Voltage -25 kV, Injection 10s x 10cm Hydrostatic, Wavelength 214nm, Temperature 25°C.

With the second electrolyte system the mobilities of the analytes were significantly less than that of the chromate probe and the equivalent-per-equivalent displacement (which would give a TR value of 0.5) was exceeded. The peak areas for the aliphatic sulfonates were 25-40% greater than would be expected for a one to one displacement based on the rules of electroneutrality. However, this does not necessarily improve the limit of detection by an equivalent amount, due to the asymmetrical character of the peaks resulting from the disparity between the mobilities of the probe and analyte. This suggests that a compromise between high TR and optimal peak shape must be attained in order to maximise sensitivity.

Table 3.2 Experimental and theoretical transfer ratios

Analyte	Experimental TR ± S.D.	Experimental TR ± S.D.*	Theoretical TR (eqn. (1.2))	Mobility 10^{-9} $m^2V^{-1}s^{-1}$ ± S.D.
Electrolyte 1[a]				
Chloride	0.97 ± 0.02	-	0.98	-77.1 ± 0.1
Sulfate	1.89 ± 0.04	1.67 ± 0.04	2.05	-68.1 ± 0.1
Cyanate	0.95 ± 0.03	0.75 ± 0.02	1.02	-63.7 ± 0.1
Formate	1.07 ± 0.08	-	1.03	-55.7 ± 0.1
Nitrate	-	-	-	-71.5 ± 0.1
Bis Tris	-	-	-	19 ± 1
Electrolyte 2[b]				
Octanesulfonate	0.73 ± 0.02	-	0.69	-25.0 ± 0.3
Heptanesulfonate	0.70 ± 0.03	-	0.68	-26.4 ± 0.3
Hexanesulfonate	0.69 ± 0.02	-	0.66	-27.9 ± 0.4
Pentanesulfonate	0.64 ± 0.02	-	0.65	-29.6 ± 0.4
Propanesulfonate	0.64 ± 0.02	-	0.62	-34.1 ± 0.3
Chromate	-	-	-	-77.2 ± 0.1
Tris	-	-	-	17 ± 1

* same conditions as for electrolyte 1 except that 0.5 m*M* TTAB was added to the electrolyte.

[a] The calibration plot of the probe for electrolyte 1, (NO_3^-) was constructed with 2.1m*M* $HClO_4$ buffered to pH 6.8 with Bis Tris.

[b] The calibration plot of the probe for electrolyte 2, ($CrO4^{2-}$) was constructed with 2.0m*M* H_2SO_4 buffered to pH 8 with Tris as the electrolyte. Chromate migrated counter to the flow of the EOF.

3.3.5 Practical Electrolyte-Analyte Systems with EOF Modifiers

In the analysis of small anions, surfactants are frequently added to reverse the direction of the EOF and thereby to enable the analysis to be conducted in a co-EOF mode with the analytes and the EOF moving towards the detector [6-8]. The surfactants used commonly are bromide salts of quaternary ammonium compounds, for example tetradecyltrimethylammonium bromide (TTAB) and cetyltrimethylammonium bromide (CTAB). Although these EOF modifiers are added typically in concentrations as low as 0.5 m*M*, the presence of the co-anion bromide provides potential competition with the probe in the detection displacement reaction and thereby a reduction in the value of TR. When 0.5 m*M* TTAB was added to the nitrate electrolyte the experimentally determined TR values for sulfate and cyanate were decreased by 12% and 21% respectively (Table 3.2).

Wang and Hartwick [5] showed that in electrolytes containing binary buffers, analytes will predominantly displace the buffer species having a mobility closest to that of the analyte. Addition of a surfactant to the electrolyte can be considered to be analogous to a binary buffer situation. Since the mobilities of bromide, nitrate, sulfate and cyanate are all similar it would be expected that sulfate and cyanate (as analytes) would displace equally nitrate and bromide (as probes). Given that bromide comprises about 20% of the total concentration of the electrolyte, it would be expected that the TR value should decrease by about 20%. This prediction is in approximate agreement with the decrease in experimentally determined transfer ratios and could partially explain the poor agreement of experimental and theoretical TR values obtained earlier [1], especially for high mobility analytes such as chloride, fluoride and sulfate when analysed with low mobility probes such as benzoate and phthalate. In these cases the bromide ion would be displaced preferentially, thereby reducing the transfer ratio significantly.

Whilst the ideal electrolyte-analyte systems used in this study show that the theoretical TR values can be obtained experimentally when using simple two-component buffered electrolytes, the practicalities of these separation systems are

limited. The nitrate electrolyte gave long migration times for slower analytes, and the chromate electrolyte produced poor peak symmetries. To make these systems more practical, an EOF modifier is required to reduce the analysis times by either suppressing or reversing the EOF. Surfactants that introduce co-anions are undesirable due to the reasons discussed previously. Consequently, CTAOH was investigated as a possible EOF modifier since its co-anion is consumed largely by the buffer and any residual hydroxide can be expected to exert little competition with the probe because of its low concentration and the fact that its mobility is much higher than that of the analytes.

Four different probes were investigated, namely benzoic acid, phthalic acid, trimellitic acid and chromic acid, each titrated with diethanolamine (DEA) to pH 9.1 (the pKa of DEA) and with 0.5 m*M* CTAOH added. This arrangement provided buffered electrolytes and a single co-anion (i.e. the probe ion). Each of the electrolytes was examined with a range of analytes having different mobilities, with TR values being calculated from eqn. (1.2) and determined experimentally. Figs. *3.3* to *3.6* show the electropherograms obtained for each separation, together with plots of TR *versus* analyte mobility for each electrolyte. In each plot the solid line indicates the theoretical TR and the points show experimentally determined values, whilst the arrow on the mobility axis marks the mobility of the probe. When the mobility of the analyte is close to that of the probe, the agreement between theory and experiment is good, but agreement diminishes as the difference in mobility between the analyte and probe increases. A strong contributor to this latter trend is peak asymmetry since TR values were determined from corrected peak areas (i.e. peak area divided by the migration time) and this correction becomes less effective and less precise as the peaks' asymmetry increases. Figs. *3.3* to *3.6* show clearly the large changes in peak symmetry that occur when different electrolytes are used.

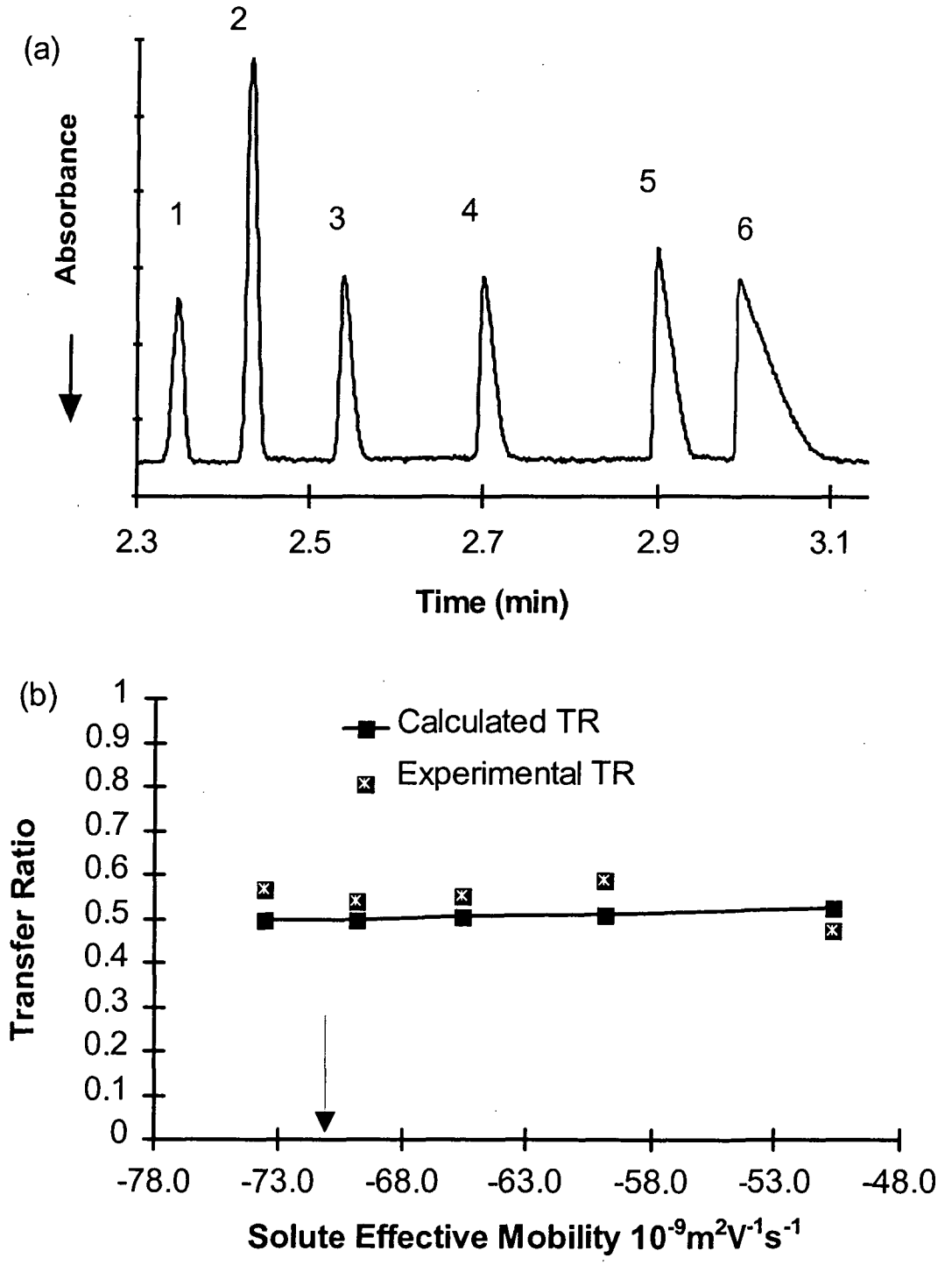


Figure 3.3 *Electropherogram (a) and plot of calculated and experimentally determined transfer ratios (b) using chromate as probe and CTAOH as EOF modifier. Conditions: electrolyte 5 mM chromic acid, 20 mM DEA, 0.5 mM CTAOH, pH 9.1, separation voltage -25 kV, hydrostatic injection at 10 cm for 10 sec, detection wavelength 254 nm, temperature 25°C, sample 0.2 mM of each ion. Key: 1 = chloride, 2 = sulfate, 3 = nitrate, 4 = cyanate, 5 = formate, 6 = citrate. The calibration plot for the probe was prepared using 5 mM H_2SO_4, 20 mM DEA, 0.5 mM TTAB at pH 9.2 as electrolyte.*

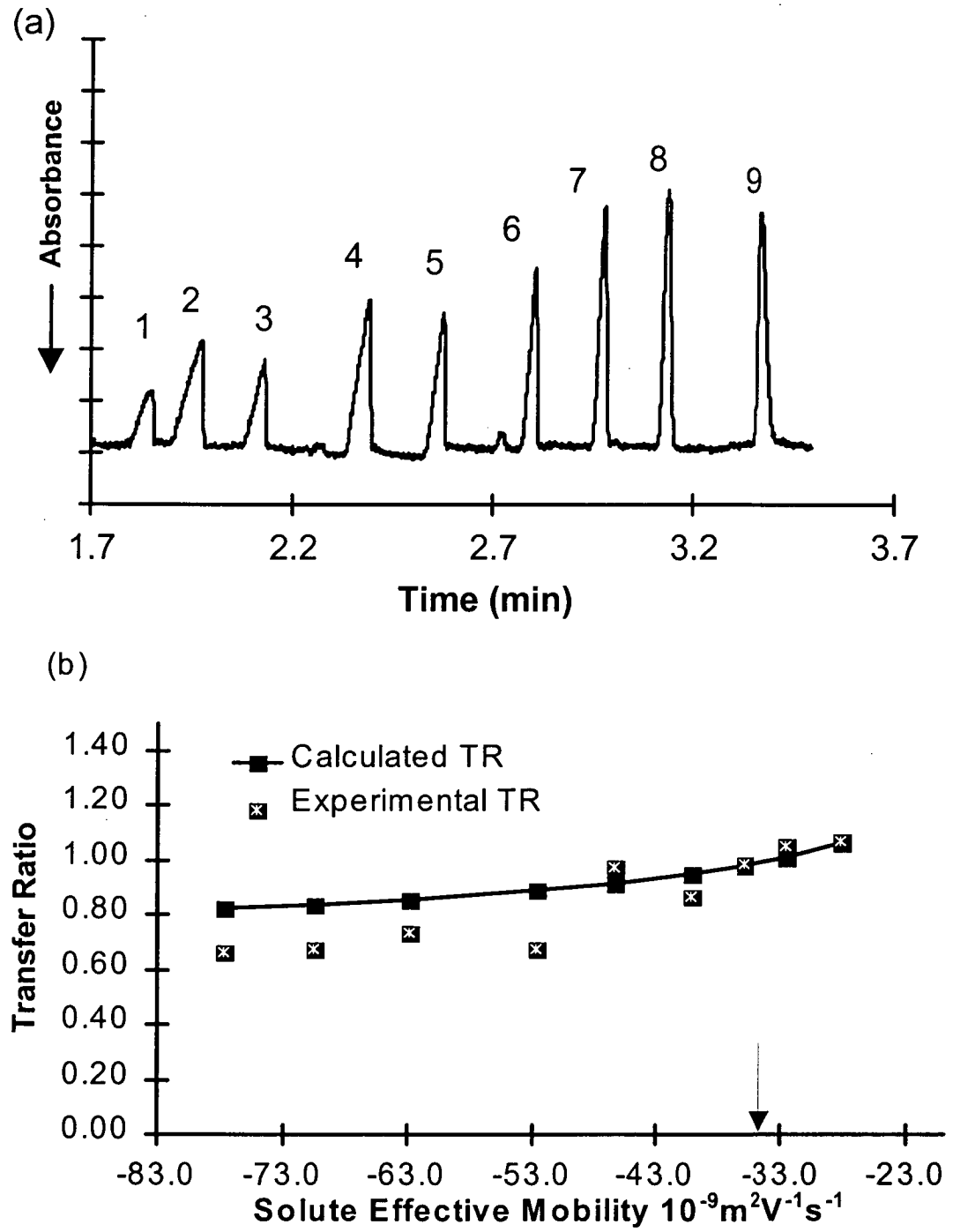


Figure 3.4 *Eectropherogram (a) and plot of calculated and experimentally determined transfer ratios (b) using a benzoate probe and CTAOH as EOF modifier. Conditions: electrolyte 10 mM benzoic acid, 20 mM DEA, 0.5 mM CTAOH, pH 9.1, separation voltage: -25 kV, hydrostatic injection at 10 cm for 10 sec, detection wavelength 254 nm, temperature 25°C, sample 0.4 mM of each ion. Key: 1 = chloride, 2 = sulfate, 3 = chlorate, 4 = phosphate, 5 = carbonate, 6 = ethanesulfonate, 7 = propanesulfonate, 8 = butanesulfonate, 9 = pentanesulfonate. The calibration plot for the probe was prepared using 10 mM methanesulfonic acid, 20 mM DEA, 0.5 mM TTAB at pH 9.1 as electrolyte.*

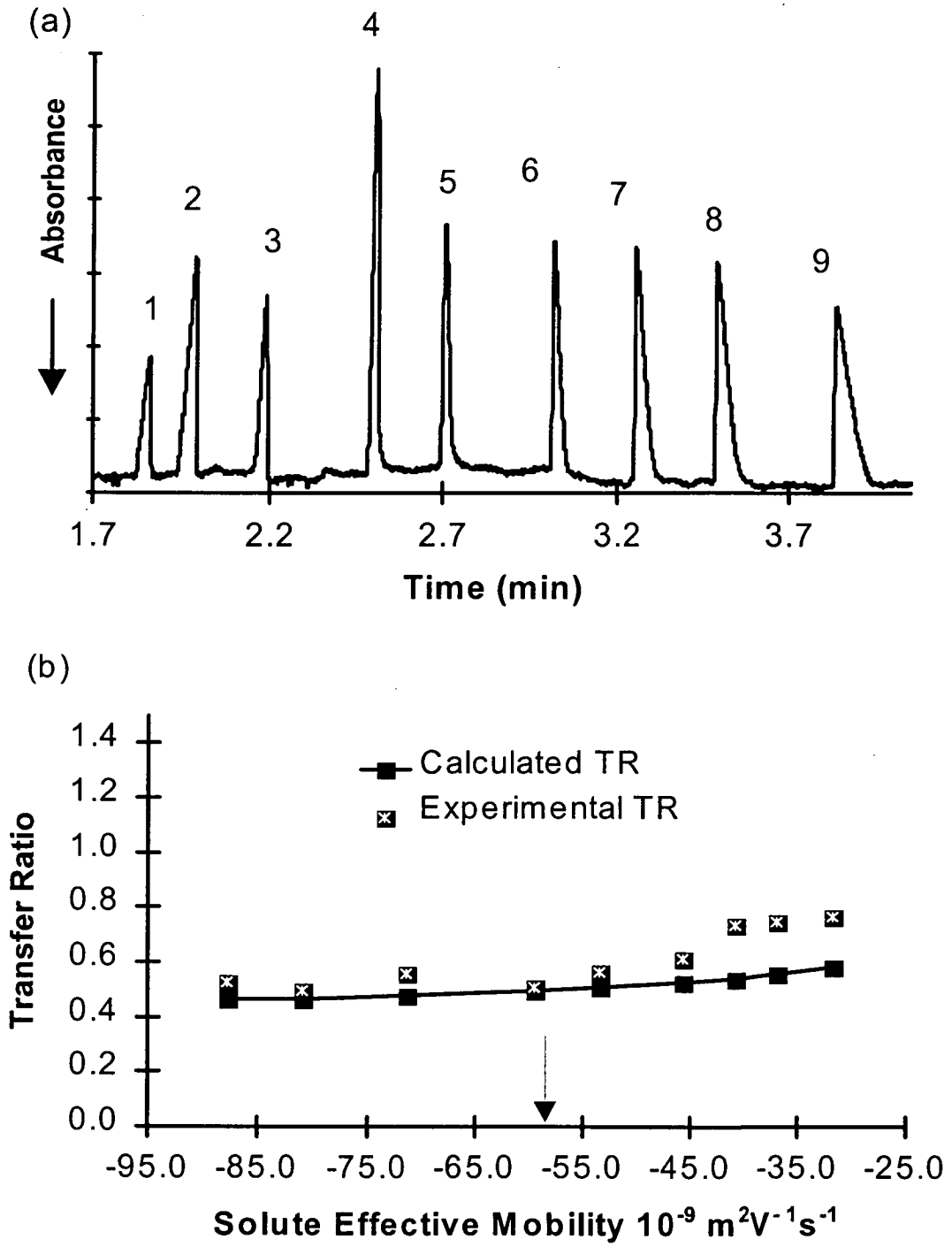


Figure 3.5 *Eectropherogram (a) and plot of calculated and experimentally determined transfer ratios (b) using a phthalate probe and CTAOH as EOF modifier. Conditions: electrolyte 10 mM phthalic acid, 40 mM DEA, 0.5 mM CTAOH, pH 9.3, separation voltage -25 kV, hydrostatic injection at 10 cm for 10 sec, detection wavelength 254 nm, temperature 25°C, sample 0.6 mM of each anion. Key: 1 = chloride, 2 = sulfate, 3 = chlorate, 4 = phosphate, 5 = carbonate, 6 = ethanesulfonate, 7 = propanesulfonate, 8 = butane sulfonate, 9 = pentanesulfonate. The calibration plot for the probe was prepared using the same electrolyte as for Figure 3.4.*

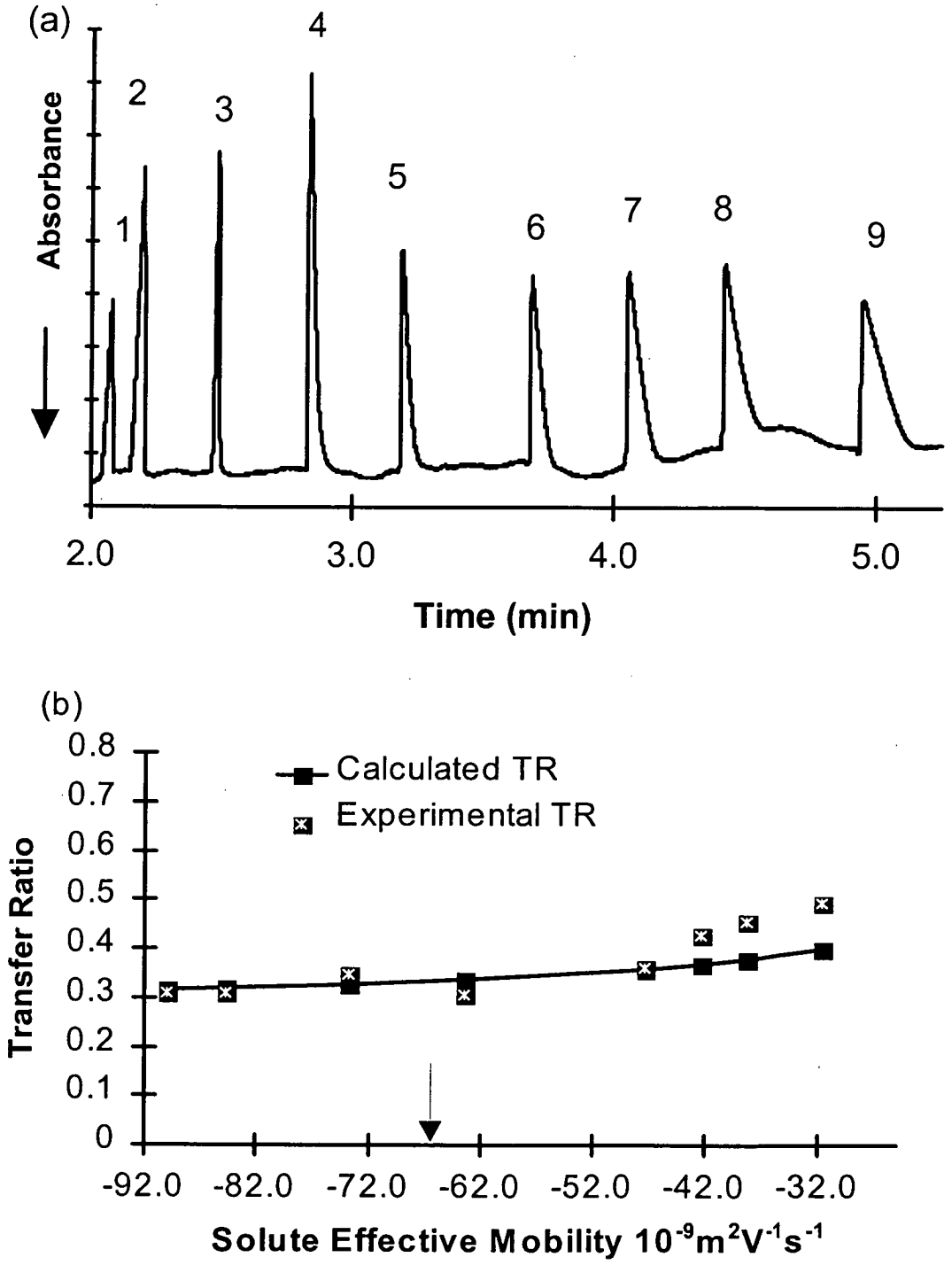


Figure 3.6 *Eectropherogram (a) and plot of calculated and experimentally determined transfer ratios (b) using a trimellitate probe and CTAOH as EOF modifier. Conditions: electrolyte 10 mM trimellitic acid, 60 mM DEA, 0.5 mM CTAOH, pH 9.3, separation voltage -25 kV, hydrostatic injection at 10 cm for 10 sec, detection wavelength 254 nm, temperature 25°C, sample 0.5 mM of each ion. Key: 1 = chloride, 2 = sulfate, 3 = chlorate, 4 = phosphate, 5 = carbonate,6 = ethanesulfonate, 7 = propane sulfonate, 8 = butanesulfonate,9 = pentanesulfonate. The calibration plot for the probe was prepared using the same electrolyte as for Figure 3.4.*

It is also evident from Figs *3.3* to *3.6* that when there is a difference between the theoretical and observed TR values, this difference is such that the experimental value is generally greater. Remembering that the chief potential drawback of using an EOF modifier was diminution of the TR value due to competition from the added co-anion, it can be seen that the strategy of using hydroxide as the co-anion introduced with the surfactant eliminates this drawback. Maximum theoretical detection sensitivity can therefore be achieved. A further advantage exists in that system peaks caused by the introduction of co-anions are eliminated. These system peaks often interfere with analyte quantification and to illustrate this point, the same nine analyte anions were separated with the benzoate probe using identical conditions to those employed previously but with TTAB as the EOF modifier (Figure *3.7*).

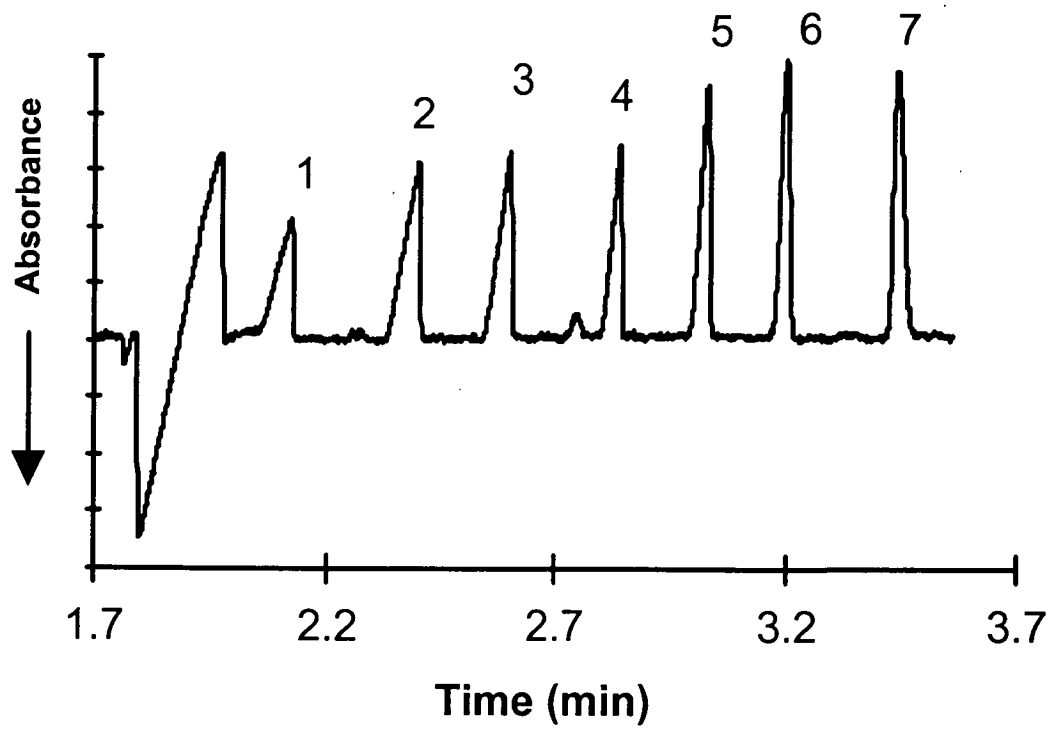


Figure 3.7 *Electropherogram obtained using a benzoate probe and TTAB as EOF modifier.Conditions: electrolyte 10 mM benzoic acid, 20 mM DEA, 0.5 mM TTAB, pH 9.1, separation voltage -25 kV, hydrostatic injection at 10 cm for 10 sec, detection wavelength 254 nm, temperature 25°C, sample 0.4 mM of each anion. Key: 1 = chlorate, 2 = phosphate, 3 = carbonate, 4 = ethanesulfonate, 5= propanesulfonate, 6 = butanesulfonate, 7 = pentanesulfonate.*

The bromide co-anion gave a negative system peak (at the migration time of bromide) which completely swamped the peaks of chloride and sulfate. In contrast, chloride and sulfate were easily quantifiable when CTAOH was used as the EOF modifier.

3.3.6 Effects of Counter-Cations on TR

As the TR value is also governed by the mobility of the counter-cation (see eqn. (1.2)), this factor must also be considered if TR is to be maximised. Figure *3.8* shows the effect on TR of chlorate (of mobility -66 x 10^{-9} $m^2V^{-1}s^{-1}$) resulting from the use of a series of probes with counter-cations of differing mobility, calculated using eqn. (1.2).

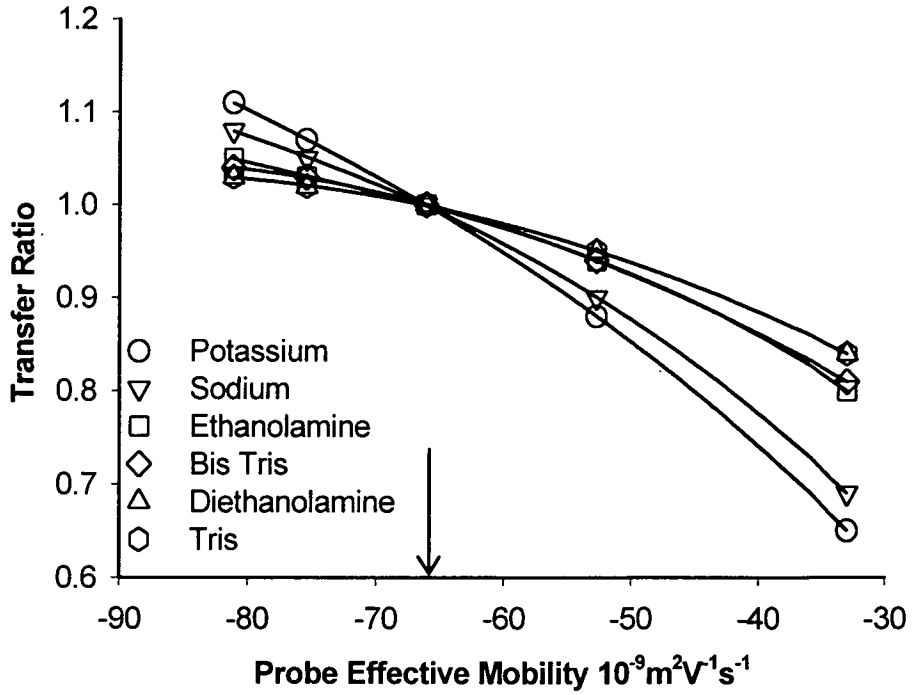


Figure 3.8 *Theoretical plot of transfer ratio for chlorate versus probe mobility for a series of counter-cations, calculated using Eqn. (1.2). The following mobilities were taken from ref [9]: chlorate -66.6x10⁻⁹m²V⁻¹s⁻¹, potassium 76.0x10⁻⁹m²V⁻¹s⁻¹, sodium 51.9x10⁻⁹m²V⁻¹s⁻¹, ethanolamine 44.3x10⁻⁹m²V⁻¹s⁻¹, protonated Tris 14.8x10⁻⁹m²V⁻¹s⁻¹. The mobility of protonated Bis Tris, 19x10⁻⁹m²V⁻¹s⁻¹, was determined experimentally. See text for details.*

A number of general interpretations can be drawn from Figure *3.8*. First, for a given counter-cation, TR for an analyte shows a general increase as the mobility of the probe approaches and then exceeds that of the analyte and this effect is most pronounced with potassium as counter-cation. Second, the TR value is unaffected by the mobility of the counter-cation when the mobilities of the probe and analyte are the same. Third, when the mobility of the analyte is less than that of the probe, the TR value is highest with more mobile counter-cations and lowest with less mobile counter-cations. Fourth, when the mobility of the analyte is greater than that of the probe the TR value is highest with less mobile counter-cations and lowest with more mobile counter-cations. Moreover, the effects of the counter-cation increase as the difference in mobility between the analyte and probe become greater. For example, the TR for the chlorate anion shown in Figure 8 is increased by 29% when benzoate (μ = -32.9 x 10^{-9} $m^2V^{-1}s^{-1}$) is used as the probe and the counter-cation is changed from potassium to protonated Tris. The TR is increased by only 8% when phthalate (μ = -52.7 x 10^{-9} $m^2V^{-1}s^{-1}$) is used as the probe and the same change in counter-cation is made.

It is also evident from Figure *3.8* that a counter-cation producing the largest enhancement of TR for an analyte of greater mobility than the probe will exert the opposite effect for an analyte of lower mobility than the probe. The best compromise for counter-cation effects on TR (and also for the production of optimal peak shapes) is therefore to ensure that the mobility of the probe is in the middle of the range of mobilities encompassed by the analytes in the particular mixture being separated. A slow counter cation is also desirable to minimise the decrease in TR for a given set of analytes.

3.4 Conclusions

The sensitivity of indirect detection can be optimised by consideration of the factors that affect TR. Maximum values of TR are obtained when the electrolyte contains a single co-anion and reproducibility is enhanced when the electrolyte is buffered. Eqn. (1.2) was shown to be valid for simple electrolyte systems that contain two

components, namely the UV absorbing probe anion and its corresponding counter-cation. Buffering of electrolytes within the above constraints can be achieved by titration of the acid form of the probe to the pKa of a basic buffer such as Bis-Tris, Tris or DEA.

The addition of a surfactant to reverse the flow of the EOF is desirable to diminish the analysis times. A surfactant that does not introduce a competing co-anion is beneficial in that sensitivity is not diminished for analytes as a result of competitive displacement and also because of the elimination of system peaks that can interfere with the quantification of analytes. This can be achieved by using a surfactant in the hydroxide form such as CTAOH. Electrolyte systems of this type show generally good agreement with eqn. (1.2), which allows TR values to be predicted.

Finally, the effects on TR of the counter-cation in the electrolyte are dependent on the relationship between the mobilities of the analyte and the probe. The best way to minimise these effects is to ensure that the mobility of the probe is in the middle of the range covered by the mobilities of the analytes, and to use a counter-cation of low mobility (such as protonated Tris or Bis-Tris).

3.5 References

[1] S. M. Cousins, P. R. Haddad and W. Buchberger, *J. Chromatogr.*, 671 (1994) 397.

[2] R. Kuhn and S. Hoffstetter-Kuhn, Capillary Electrophoresis: Principles and Practice, Springer Laboratory, 1993.

[3] F. Foret, S. Fanali, L. Ossicini and P. Bocek, *J. Chromatogr.*, 470 (1989) 299.

[4] N. Guzman, Capillary Electrophoresis Technology, Marcel Dekker Inc, New York, 1993.

[5] T. Wang and R. A. Hartwick, *J. Chromatogr.*, 589 (1992) 307.

[6] J. Romano, P. Jandik, W.R, and P.E. Jackson, *J. Chromatogr.*, 546 (1991) 411.

[7] B.J. Wildmam, P.E. Jackson, W.R. Jones, and P.G. Alden, *J. Chromatogr.*, 546 (1991) 459.

[8] W.R. Jones, and P. Jandik, *J. Chromatogr.*, 608 (1991) 385.

[9] J. Pospichal, P. Gebauer, and P. Bocek, *Chem Review.*, 89 (1989) 419.

4. Use of Electrolytes containing Multiple Probe Ions for the Analysis of Anions

4.1 Introduction

Most determinations of anions using indirect detection have been performed with BGEs that consist of two components, the probe and its counter cation, under which conditions the displacement process is simple and well understood [1,2]. However there are several drawbacks when such BGEs are used. These include a limited range of pH of the BGE since any buffering is limited to the pH range close to the pKa of the probe, poor buffering capacities, and poor peak shapes due to differences in mobility between the analytes and the probe. Optimum peak shapes and highest sensitivity for any given BGE are achieved by matching the mobilities of the probe and the analytes, with the best results being obtained when these mobilities are equal [3]. Peaks of analytes with faster mobilities than the probe have a fronted profile, whilst the peaks of analytes with slower mobility exhibit a tailed profile.

The purpose of the present chapter was to investigate the effect of BGEs containing multiple co-anions on sensitivity (and also peak symmetries) in the determination of anions using indirect detection, and to gain a greater understanding of the displacement process occurring in such BGEs. Initially, co-anions which do not absorb at the detection wavelength are added to a BGE, with phthalic acid as the probe, buffered with diethanolamine (DEA) at its *pKa* of 9.2 . Two cases are considered. The first is addition of co-anions having a greater mobility than the probe, and the second is addition of co-anions having a lower mobility than the probe. BGEs containing two or three probes are then investigated as a means to improve peak symmetries and methods for optimizing the composition of BGEs containing multiple probes are determined. Finally, the question of system peaks is addressed and a simple procedure proposed for the prediction of the mobility of the system peak based on the mobility of the co-anions and their relative concentrations.

4.2 Experimental

The general experimental is given in Chapter Two. Detailed conditions are included in each of the figure captions.

4.3 Results and Discussion

4.3.1 BGEs with Added Co-Anions Having Higher Mobility than the Probe

Initial experiments were undertaken to investigate the effects of addition to the BGE of co-anions that do not absorb at the detection wavelength on the peak shape and area for analyte anions having a range of mobilities. The first case considered was the addition of co-anions having a higher mobility than the probe, with chloride being chosen as the non-absorbing co-anion and phthalate as the probe. Mobilities for all analytes and probes used for this study are given in Table 4.1. Calibration curves for analyte anions within the mobility range of -78 to -10 ($10^{-9}m^2V^{-1}s^{-1}$) were constructed with four BGEs using duplicate injections of 0.1, 0.2, 0.3, and 0.6 m*M* of each analyte anion. The first BGE (BGE 1 in Table 4.2) contained phthalic acid buffered with DEA to the pKa of DEA; i.e., a BGE having only a single co-anion . The remaining three BGEs (BGEs 2-4 in Table 4.2) all contained sequentially increasing concentrations of the added co-anion, chloride, but with the total ionic strength maintained at a constant value. The calibration curves constructed for all four BGEs were used to generate a plot of response ratio *versus* analyte mobility. The response ratio is defined as the slope of the calibration curve generated using the multiple co-anion BGE divided by the slope of the calibration curve obtained using the single co-anion BGE (BGE 1). A response ratio of unity would occur if the peak areas were unaffected by the addition of the chloride co-anion, of less than unity if the peak areas were diminished, and of greater than unity if the peak areas were enhanced. Intuitively, one would expect the peak areas to be reduced by the addition of UV competing co-anions due to competition between the probe and the co-anion for displacement by the analyte. However, the response ratio was found to be greater

Table 4.1 Mobilities of Analytes and Probes

Analyte	Mobility $10^{-9}m^2V^{-1}s^{-1}$	Probe [a]	Mobility [b] $10^{-9}m^2V^{-1}s^{-1}$
Chloride	-76.1	Chromate	-73.4
Sulfate	-73.6	Trimellitate	-66.1
Chlorate	-63.3	Sulfophthalate	-60.7
Carbonate	-47.1	Napthalenedisulfonate	-
Methanesulfonate	-45.1	Phthalate	-43.8
Ethanesulfonate	-39.7	Hydroxybenzenesulfonate	-41.6
Propanesulfonate	-35.6	Hydroxybenzoate	-32.3
Butanesulfonate	-32.4	Benzoate	-29.3
Pentanesulfonate	-28.6	Toluensulfonate	-29.3
Hexanesulfonate	-22.3		
Heptanesulfonate	-9.6		
Propanoate	-35.0		

[a] All BGEs were prepared from the acid form of the probe and buffered to a final concentration of 20 m*M* DEA. 0.5 m*M* TTAOH was added to each as the EOF modifier.

[b] Probe mobilities were determined experimentally. BGE: 10 m*M* HCl, 20 m*M* DEA, 0.5 m*M* TTAOH, pH=9.35. Conditions: -25 kV, 10cm x 10 sec injection of 0.2 m*M* of each probe, detection at 254nm.

Table 4.2 BGE compositions for two-probe BGEs

BGE	Phthalic Acid (m*M*)	HCl (m*M*)	Propanoic Acid (m*M*)	DEA (m*M*)	TTAOH (m*M*)	pH
1	10.0	-	-	40	0.5	9.28
2	7.5	5.0	-	40	0.5	9.27
3	5.0	10.0	-	40	0.5	9.24
4	2.5	15.0	-	40	0.5	9.22
5	7.5	-	5.0	40	0.5	9.24
6	5.0	-	10.0	40	0.5	9.23
7	2.5	-	15.0	40	0.5	9.25

than unity, unity or less than unity depending upon the relative mobilities of the analyte, the probe and the competing co-anion.

Figure *4.1* presents the response ratio plot (Figure *4.1(a)*), and the associated electropherograms (Figure *4.1(b)*) for the series of analyte anions obtained using the four BGEs. The electropherogram obtained with phthalate alone (BGE 1) exhibited the typical profile expected. Peaks 1-3, which have a higher mobility than the probe, showed a fronted profile. Peak 4 which has approximately the same mobility as the probe was symmetrical, whilst peaks 5-10 which were all due to analytes with lower mobility than the probe gave a tailed profile. Addition of chloride to the BGE produced some unexpected results.

When the concentration ratio of chloride to phthalate was 5 m*M*: 7.5 m*M* (BGE 2), peak 1 (chloride) gave a negative detector response (i.e. an increase in absorbance

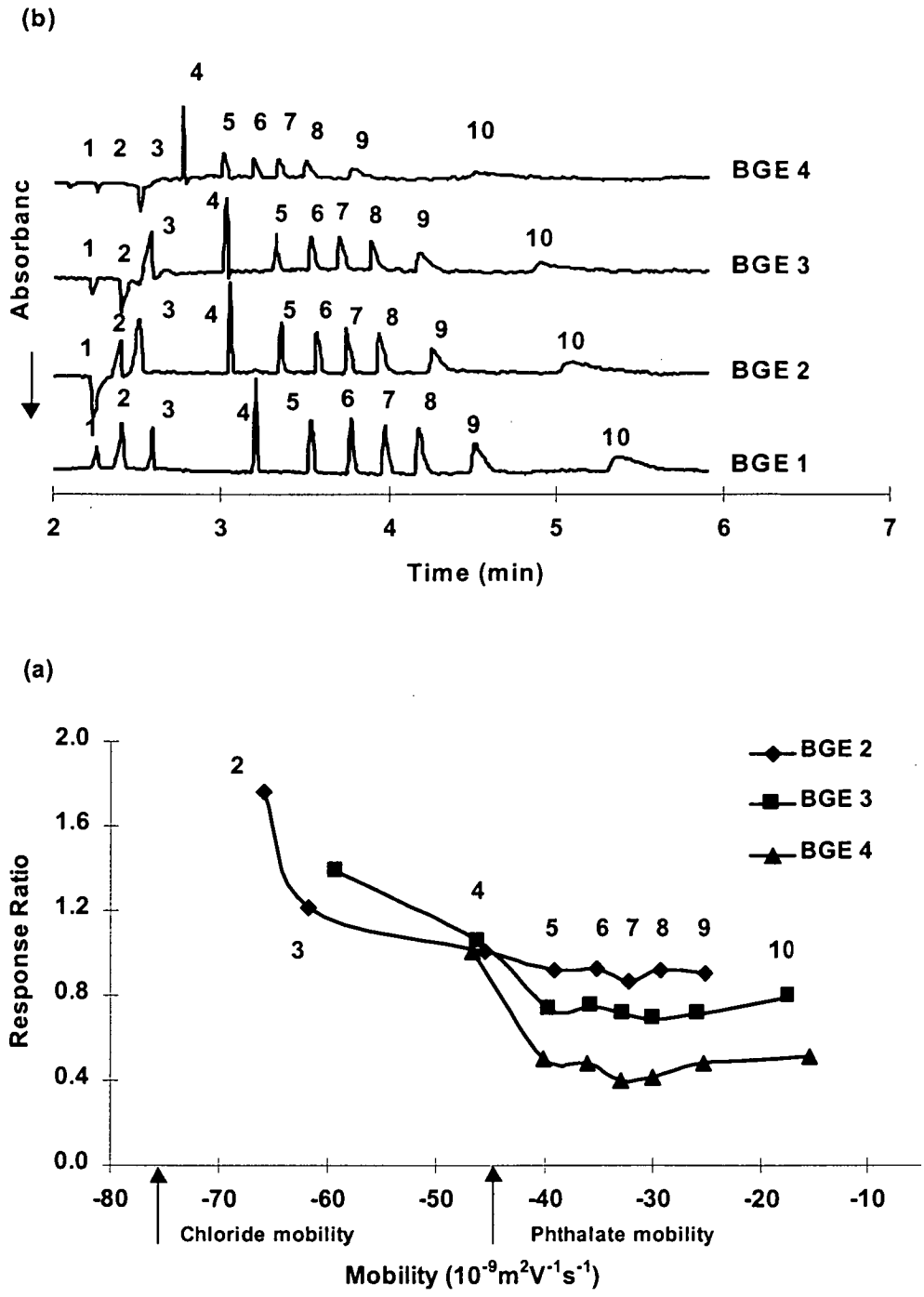


Figure 4.1 *Plot of response ratio (see text) versus analyte mobility (a) and electropherograms (b) for BGEs containing an added co-anion of high mobility (chloride). The arrows on the horizontal axis of (a) mark the mobilities of chloride and phthalate. Conditions: Separation voltage -20 kV, hydrostatic injection at 10 cm for 10 sec, detection wavelength 254 nm, temperature 25°C. Key: 1 = chloride, 2 = sulfate, 3 = chlorate, 4 = methanesulfonate, 5 = ethanesulfonate, 6 = propanesulfonate, 7 = butanesulfonate, 8= pentanesulfonate, 9 = hexanesulfonate, 10 = heptanesulfonate.*

because the detector polarity was such that indirect detection peaks appeared in the positive direction) and its peak symmetry was reversed in comparison to BGE 1; i.e. the peak profile was tailed rather than fronted. It should be noted here that peaks that gave a negative detector response have been omitted from the response ratio *versus* mobility plot shown in Figure *4.1(a)*. Peaks 2 (sulfate) and 3 (chlorate) showed the same symmetry as for BGE 1 but their responses were increased by factors of 1.76 and 1.22, respectively. When the concentration ratio of chloride to phthalate was increased to 10 m*M*: 5 m*M* (BGE 3), peak 2 gave a negative detector response and its symmetry was reversed in the same manner as discussed above for peak 1 in BGE 2. Peak 3 was further increased in area by a factor of 1.38 compared to the single co-anion BGE. Further increase of the concentration ratio of chloride to phthalate to 15 m*M*: 2.5 m*M* (BGE 4) again caused a negative detector response for peak 3 and reversal of symmetry. One explanation for the observed reversal of symmetry for peaks 1, 2 and 3 was that these analytes were preferentially displacing chloride at the expense of phthalate, and the extent of this displacement was dependent upon the relative concentration of chloride and phthalate in the BGE.

The response ratio of peak 4 (methanesulfonate) was close to unity with BGEs 2-4 and its peak symmetry was relatively unaffected by changes in the BGE composition. This in agreement with earlier findings [4] that analytes will mainly displace the probe ion with the closest mobility to that of the analyte. Methanesulfonate has almost the same mobility as phthalate and preferentially displaced phthalate, so the detector response was independent of the concentration of chloride or phthalate in the BGE.

The symmetries of peaks 5-10 were also relatively unaffected by the addition of chloride, but the response ratios diminished with increasing concentration of chloride. When analytes have lower mobilities than both the probe and the added co-anion, competitive displacement (i.e. both the probe and the co-anion are displaced by the analyte anion) occurs but there is still evidence of preferential displacement of

the probe. If no discrimination of displacement occurred the response ratios would decrease in proportion to the addition of chloride. For example, BGE 4 contained a ratio of 2.5 m*M* phthalate: 15 m*M* chloride and accounting for the double negative charge on phthalate it can be calculated that phthalate contributes 25% of the charge available for displacement and chloride 75%. Thus it would be expected that the response ratio would decrease by 75% if the analyte exhibited no preferential displacement, whereas the observed response ratios were decreased by approximately 50%. The most plausible reason for this preferential displacement is that the mobility of phthalate is much closer to those of the analytes in question than is that of chloride.

A number of general interpretations can be drawn from the response ratio plot for the addition of non-absorbing co-anions that have a higher mobility than the probe. First, analytes that migrated between the co-anion and the probe gave either negative detector response or were increased in area, as determined by the relative concentration of the co-anion and the probe. When the analyte gave a negative detector response the peak symmetry was reversed compared to that evident in a BGE with a single co-anion. Second, peak symmetry and detector response of analytes having the same mobility as the probe were unaffected by the addition of co-anions. Third, for analytes with mobility less than that of the probe, peak symmetries were unaffected by the addition of a co-anion to the BGE but detector response diminished with increasing concentration of added co-anion, although there was still a preference for the analytes to displace the BGE component having the closest mobility to that of the analyte.

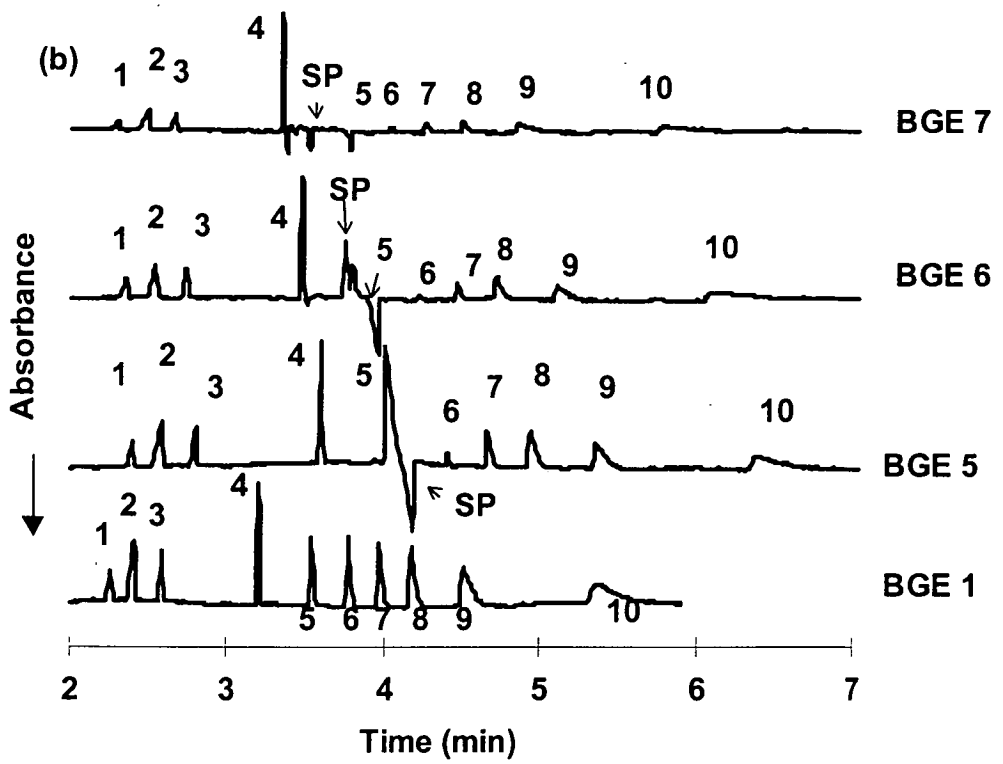
The addition of buffers to BGEs used for indirect detection is often viewed as desirable in order to stabilise migration times. It is apparent from the above results that the addition of buffers which introduce co-ions of higher mobility than the probe is not suitable because deleterious effects are observed for virtually all analytes, regardless of their mobilities.

4.3.2 BGEs with Added Co-Anions Having Lower Mobility than the Probe

The second case considered was the effect of addition to the BGE of co-anions having a lower mobility than that of the probe. Again, phthalate was chosen as the probe, with propanoate as the co-anion. Response ratios of the same anions were calculated using the same process as before. Figure *4.2* shows the response ratio plot (Figure *4.2(a)*), and the associated electropherograms (Figure *4.2(b)*). Addition of propanoate to the BGE (BGEs 5-7 in Table 4.1) resulted in the appearance of a system peak that migrated with a mobility between those of phthalate and propanoate, with the exact migration time being dependent upon the concentration ratio of phthalate to propanoate.

In general, increasing the concentration of propanoate caused the system peak to migrate closer to the mobility of phthalate, and increasing the concentration of phthalate resulted in the system peak migrating closer to the mobility of propanoate.

Peaks 1-3 all migrated well before the system peak and their response ratios diminished with increasing concentration of propanoate. However, the response ratios did not decrease proportionally to the concentration of added propanoate, again indicating that these analytes preferentially displaced phthalate. This is the same effect observed above for peaks 5-10 when the faster co-anion was added to the BGE. Again, the analytes were preferentially displacing the BGE component with mobility closest to that of the analyte. Peaks 4 and 5 migrated in close proximity to the system peak and response ratios for both species were increased, with the detector response and peak symmetry for peak 5 being reversed in BGEs 6 and 7. The detector response of the system varied between BGEs. The response ratios of peaks 6-10 decreased significantly with increasing concentration of propanoate, with the most pronounced effect being evident for peak 6 (propanesulfonate) which has almost the same mobility as propanoate. Analytes 6-10 have mobilities closer to propanoate than phthalate and therefore preferentially displace the non-absorbing co-anion.



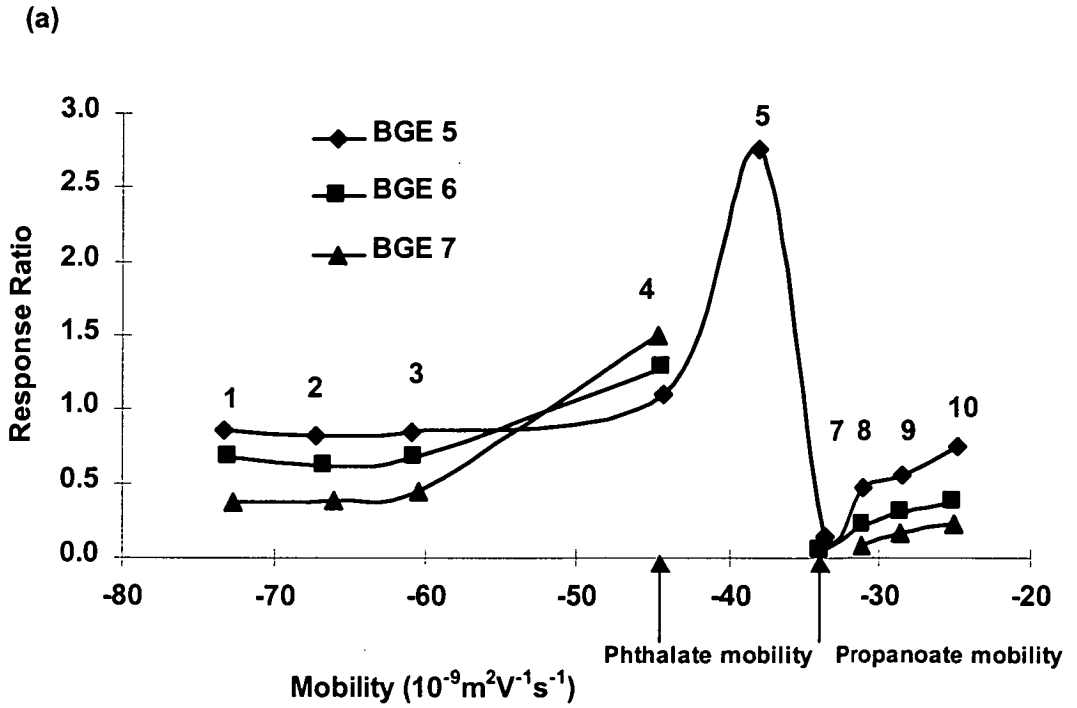


Figure 4.2 *Plot of response ratio (see text) versus analyte mobility (a) and electropherograms (b) for BGEs containing an added co-anion of low mobility (propanoate). The arrows on the horizontal axis of (a) mark the mobilities of phthalate and propanoate. Conditions and analyte identities as for Figure 4.1, SP = system peak*

Again, a number of general interpretations can be drawn from the response ratio plot for the addition of non-absorbing co-anions having a lower mobility than the probe. First, analytes that migrated well before the system peak showed diminished detector responses with increasing concentration of the non-absorbing co-anion in the BGE, but still preferentially displaced the probe. Second, analytes that migrated just before the system peak gave enhanced detector response and this effect became more pronounced with increasing concentrations of the co-anion. Third, analytes that migrated just after the system peak and before the mobility of the co-anion will be negative in response, and reverse their symmetry. Fourth, analytes that migrated at or after the mobility of the co-anion gave dramatically reduced detector responses due to preferential displacement of the co-anion.

Unlike the addition of fast co-anions, slower co-anions may be added at low concentration to the BGE when indirect detection is to be used so as to provide buffering for a probe having poor or no buffering capacity. A BGE consisting of a probe of fast to intermediate mobility could be used with a slow moving buffer co-ion such as borate or CHES. In such a situation, minimal loss of sensitivity would occur for analyte anions with similar mobility to the probe. In addition, it would be desirable to keep the ratio of concentrations of the probe and buffer as high as possible to move the system peak away from analytes of interest.

4.3.3 BGEs with Two Probe Co-Anions

The preferential displacement behaviour exhibited above can be utilised to design BGEs that contain multiple probes. The same mixture of analyte anions was used to investigate this possibility using a BGE containing two probe co-anions. Figure *4.3(a)* shows a plot of the slope of the calibration curves *versus* analyte mobility for BGEs containing phthalate, chromate or an equimolar mixture of chromate and phthalate. Figure *4.3(b)* shows the associated electropherograms. Figure *4.4* shows the variation of the tailing factor (expressed on a logarithmic scale) with analyte mobility for all three of the BGEs.

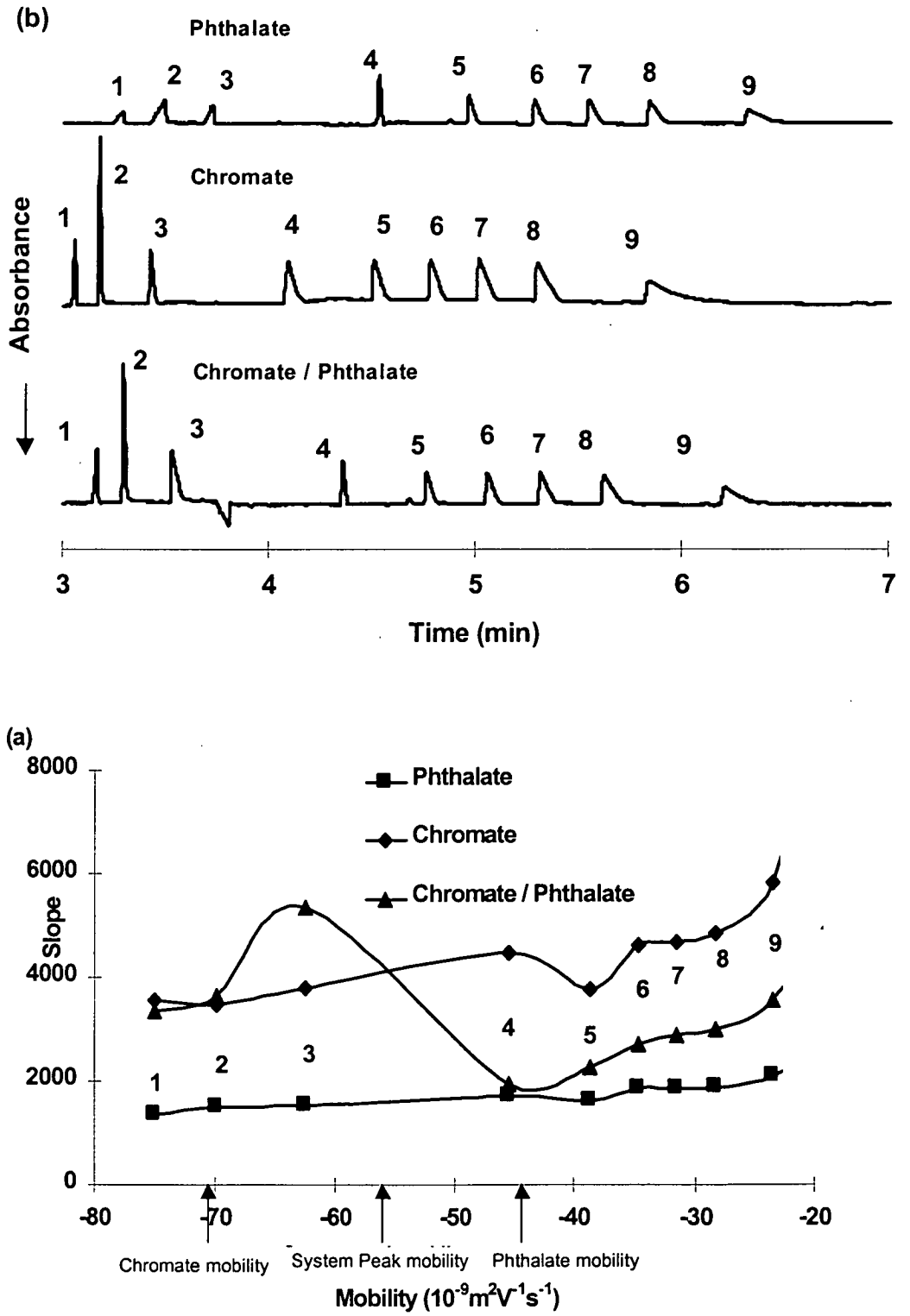


Figure 4.3 *Slope of calibration curve slope versus analyte mobility (a) and electropherograms (b) for BGEs containing chromate, phthalate, or a mixture of chromate and phthalate as probes. The arrows on the horizontal axis of (a) mark the mobilities of chromate, phthalate and the system peak. Conditions and analyte identities as for Figure 4.1.*

The tailing factor (T_f) is defined as:

$$T_f = \frac{w}{2f} \qquad (4.1)$$

where w = width of the peak at the baseline, and f = width of the first half of the peak Thus peaks with a $T_f < 1$ have a fronted profile, a value of one indicates a symmetrical peak, and $T_f > 1$ indicates a tailed peak profile. Peaks 1, 2 and 3 show significant fronting when the low mobility probe phthalate is used alone. These peaks become more symmetrical when the faster moving chromate is used as probe, and the symmetry is maintained with the mixture of chromate and phthalate since the analytes preferentially displace the probe to which their mobility is closest, ie. the chromate ion.

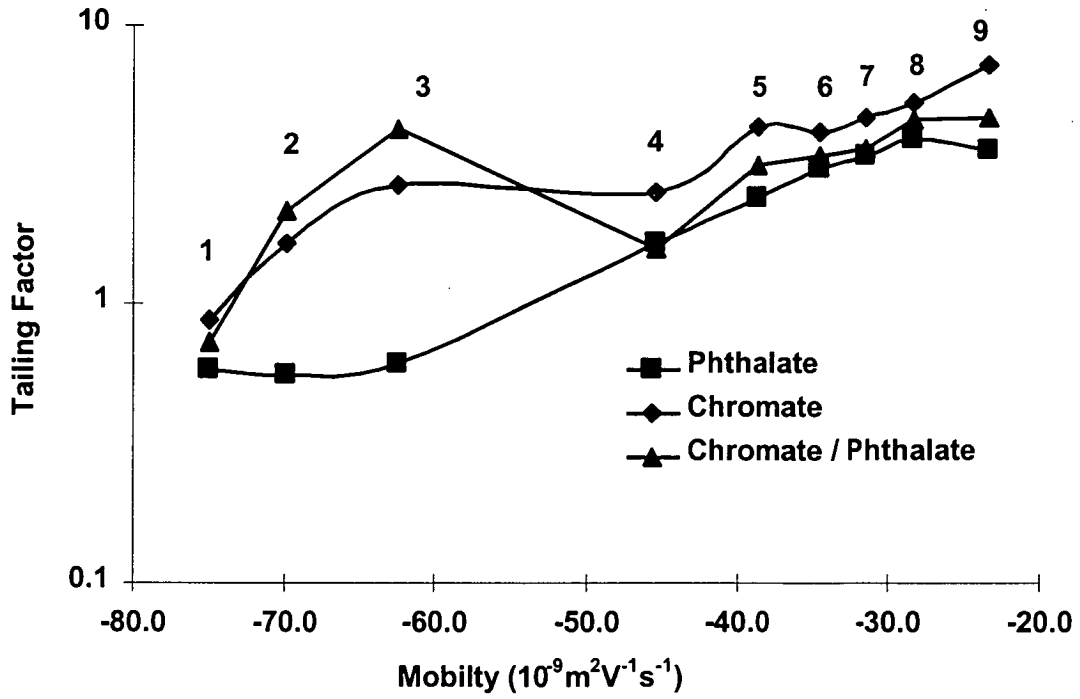


Figure 4.4 *Tailing factor as a function of analyte mobility for BGEs containing chromate, phthalate, or a mixture of chromate and phthalate as probes. Conditions and analyte identities as for Figure 4.1*

This is confirmed in Figure *4.3(a)* by comparison of the calibration slopes obtained with the chromate BGE and the mixture of the two probes, which shows that the slopes for peaks 1 and 2 were unchanged when phthalate was added to a chromate BGE. On the other hand, the slope for peak 3 was greater with the mixture than the single probe, which resulted from the same processes observed earlier for analytes migrating just before the system peak when non-absorbing co-anions were added to the BGE.

For the remaining peaks (4-9) the behaviour exhibited for the mixed probe BGE was a hybrid of the two single probe BGEs. Peak 4 has similar mobility to phthalate and its behaviour was relatively unaffected by the addition of the chromate probe co-anion. On the other hand, peaks 5-9 all have slower mobilities than both of the probes and the slopes and T_f values of these peaks in the two-probe BGE were closer to those obtained with the phthalate BGE than for the chromate BGE. This indicated that these peaks were preferentially displacing phthalate, resulting in improved peak symmetries than obtained when chromate alone was used as the probe.

4.3.4 BGEs with Three Probe Co-Anions

The above approach can be further extended to the use of three probes in order to increase the useful mobility range of the BGE. Figure *4.5* shows an electropherogram of 14 analytes obtained with a BGE consisting of chromate, phthalate and benzoate. Chromate has the highest mobility, phthalate has intermediate mobility and benzoate has the lowest mobility. Introduction of a third probe results in the appearance of a second system peak. Thus for any BGE of n probes there will be n-1 system peaks.

It can be seen that the electropherogram consisted of three distinct mobility segments corresponding to each probe in the BGE. Peaks 1-7 migrated in the first mobility segment and were mainly displacing chromate. Peaks 8-10, which migrated after the first system peak and before the second system peak, comprised the second mobility

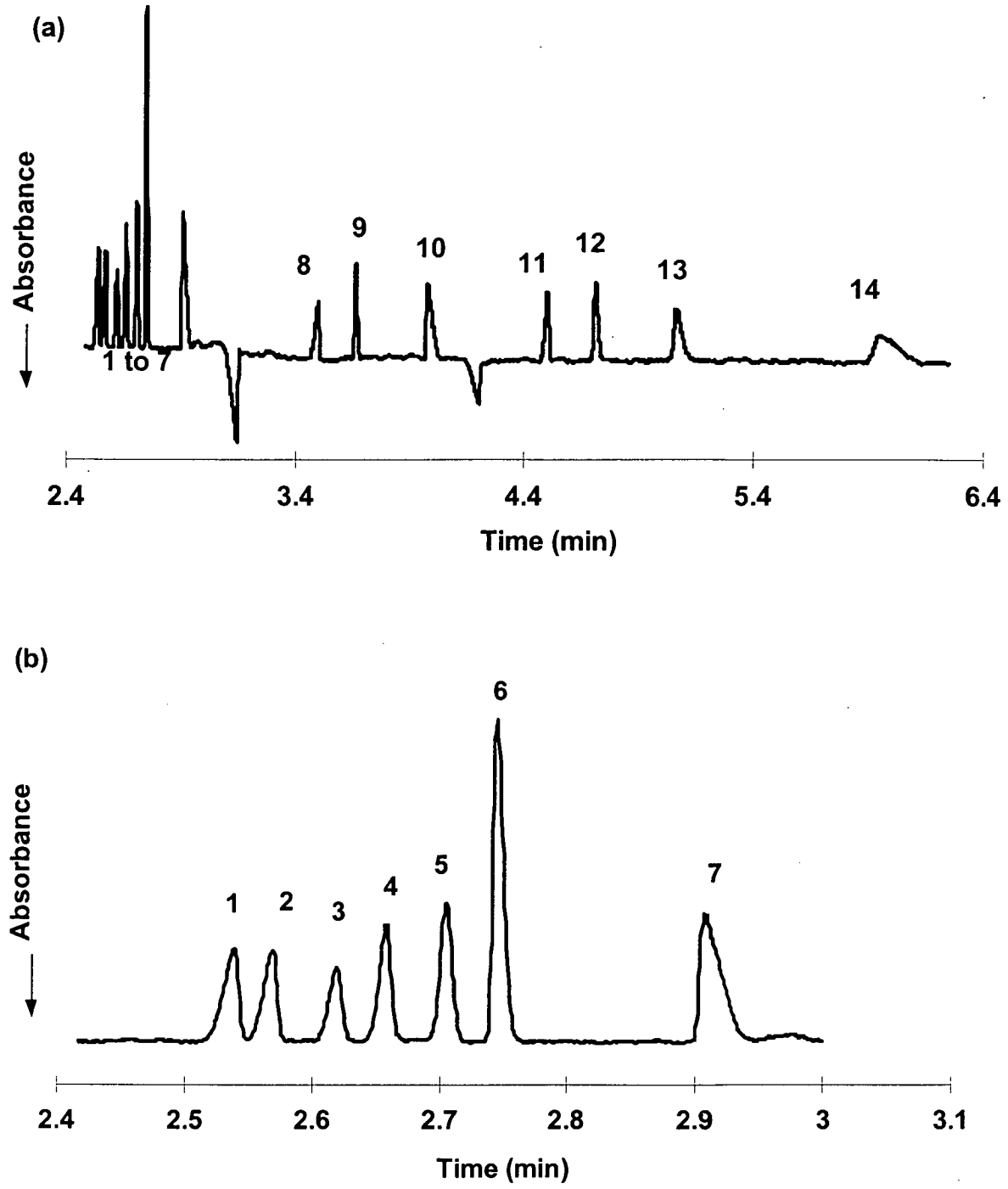


Figure 4.5 *Electropherogram obtained using a BGE with three probes (a) and zoom view (b). Conditions: BGE: 5.0 mM chromic acid, 5.0 mM phthalic acid, 10 mM benzoic acid, 60 mM DEA, 0.5 mM TTAOH, pH 9.20. Separation voltage -20 kV, hydrostatic injection at 10 cm for 10 sec, detection wavelength 254 nm, temperature 25°C. Key: 1 = bromide, 2 = chloride, 3 = iodide, 4 = nitrite, 5 = nitrate, 6 = sulfate, 7 = chlorate, 8 = phosphate, 9 = carbonate, 10 = ethanesulfonate, 11 = butanesulfonate, 12 = pentanesulfonate, 13 = hexanesulfonate, 14 = heptanesulfonate. The sample mixture contained 0.3 mM of each anion.*

segment and were mainly displacing phthalate. Peaks 11-14 migrated after the second system peak and comprised the third mobility segment, mainly displacing benzoate. The symmetry profiles for each of the three segments mirrored that obtained when using the probes alone.

4.3.5 System Peaks

A system peak can be considered any peak that does not correspond to a sample component and is due to a discrete zone migrating through the capillary. The production of system peaks has been described mathematically by Poppe [5] who discussed the behaviour of a multicomponent electrophoretic system in terms of an eigenvalue problem. Practical rules for predicting the existence of system peaks have recently been reported by Macka *et al.* [6], who proposed a descriptive model based on transient isotachophoresis of the sample species and of the co-anion from the BGE that has the closest mobility to that of the analyte ion. Williams and Vigh [7] developed generalised peak shape rules for prediction of the peak shapes of the analyte, co-ions and counter-ions with direct U.V. detection. They report that analytes that migrate on or very close to system peaks have abnormal shapes or can show no response. It is desirable to know the mobility of the system peak to avoid possible interference between the system peak and an analyte of interest. So far, no studies have provided a way to achieve this.

A two-probe BGE consisting of chromate and phthalate was chosen to determine the mobility of the system peak at different mole fractions of each of the probe at a constant ionic strength. A series of 9 BGEs was prepared (BGEs 8-16, Table 4.3) in which the mole fraction of chromate was increased sequentially by 0.1. The system peak was induced by injection of a 0.1 m*M* chloride standard solution for BGEs comprising 0.1- 0.5 mole fraction of chromate. For BGEs comprising 0.6-0.9 mole fraction of chromate, it was necessary to induce the system peak with the less mobile analyte anions, sulfate and chlorate since chloride did not give an observable system peak. The mobility of the system peak was calculated for all BGE compositions (Table 4.3). The system peak always migrated at a mobility between those of the

two probes and its exact position was dependent upon the relative concentrations of each probe. Increasing the mole fraction of chromate moved the mobility of the

Table 4.3 BGE compositions and system peak mobility data used in the study of system peaks with two-probe BGEs

BGE*	Chromate (m*M*)	Phthalic acid (m*M*)	Mole fraction of Chromate	Experimental system peak mobility (10^{-9} $m^2V^{-1}s^{-1}$)	Calculated system peak mobility (10^{-9} $m^2V^{-1}s^{-1}$)
8	0.5	4.5	0.1	-69.5	-70.8
9	1.0	4.0	0.2	-66.4	-68.2
10	1.5	3.5	0.3	-64.1	-65.6
11	2.0	3.0	0.4	-61.7	-63.0
12	2.5	2.5	0.5	-59.3	-60.5
13	3.0	2.0	0.6	-56.6	-57.9
14	3.5	1.5	0.7	-54.8	-55.3
15	4.0	1.0	0.8	-52.7	-52.7
16	4.5	0.5	0.9	-50.6	-50.1

*All BGEs were buffered with 40 m*M* DEA at pH=9.2, with 0.5 m*M* TTAOH added as EOF modifier.

system peak away from the mobility of chromate and closer towards the mobility of phthalate. The experimental system peak mobility data when plotted against the mole fraction of chromate in the BGE yielded a linear relationship.

It is possible to relate the mobility of the system peak to the mobility and relative concentration of each of the two probes in the BGE. The above data may be empirically fitted to a linear equation of the form:

$$\mu_s = (\mu_{p1} - \mu_{p2})x - \mu_{p1} \qquad (4.2)$$

where: μ_s = mobility of the system peak, μ_{p1} = mobility of probe 1 (fast probe), μ_{p2} = mobility of probe 2 (slow probe), and x = mole fraction of probe 1. The mobilities of each of the probes was determined experimentally (Table 4.3) and the mobility of the system peak was calculated for each BGE composition using eqn. (4.2). The results, listed in Table 4.3, show good agreement between the calculated and experimentally determined system peak mobility, indicating that this method can be used to calculate the position of the system peak and to provide a possible means to tailor the probe concentration ratio in the BGE to avoid any interference of a particular analyte of interest.

4.3.6 *Optimisation of the Composition of BGEs with Multiple Probe Co-Anions*

An ideal multiple probe BGE for the determination of a mixture of analyte anions having a wide mobility range would contain a series of probes that approximately match the mobility of all of the analytes. However, an additional system peak is introduced with each additional probe and each system peak increases the possibility of interference with the analytes. Consequently, the optimum multiple probe BGE would consist of the minimum number of probes that provides acceptable peak shape and detector response for each of the analytes. Accordingly, a series of probes was investigated with 10 analytes that incorporated the same mobility range as before (i.e. -76 to -10 x $10^{-9}m^2V^{-1}s^{-1}$). Each BGE was maintained at the same ionic strength and the analytes were run with each of the probes. Peak symmetries, detection sensitivities, and mobility ranges were compared. Normalised peak heights divided

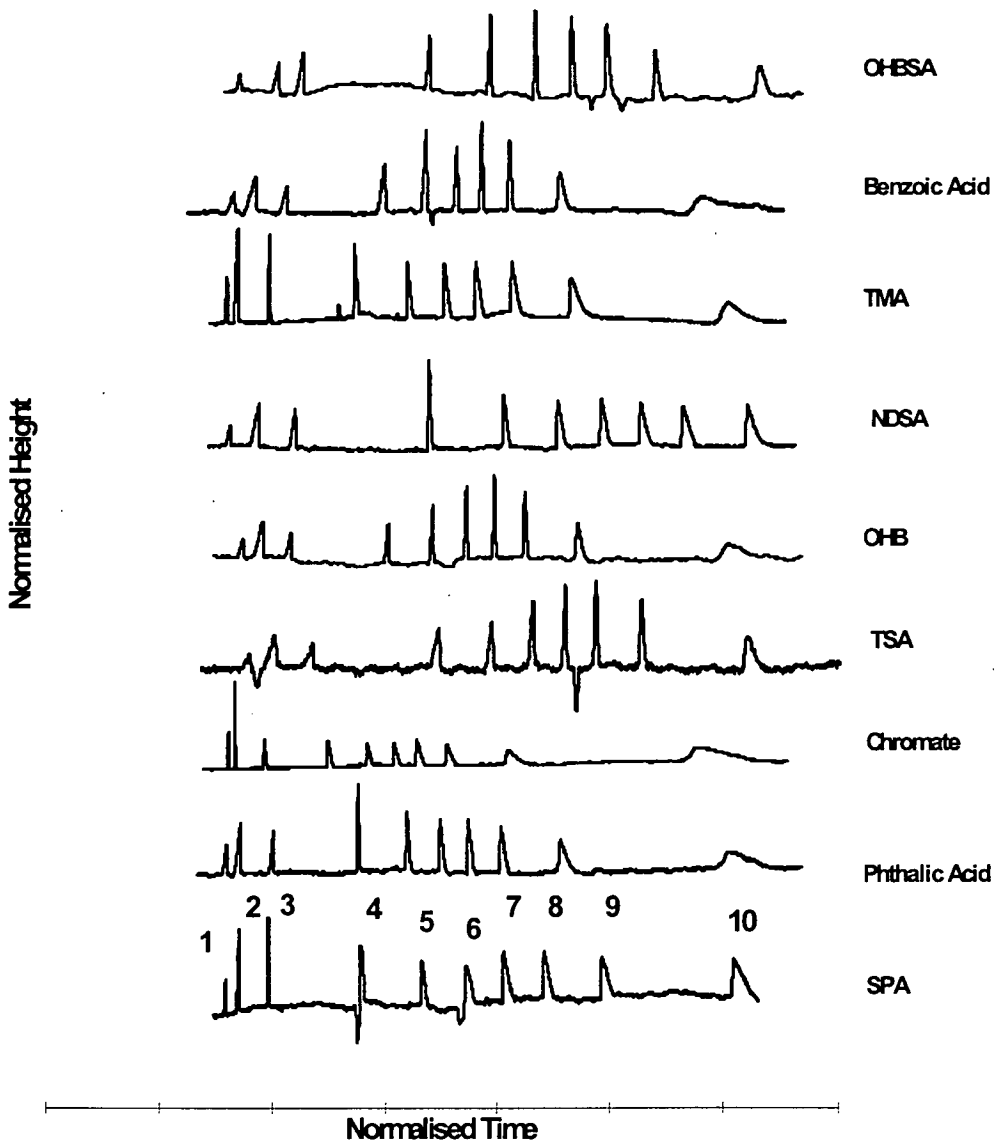


Figure 4.6 *Electropherograms obtained using BGEs with a range of different probes. Conditions: BGEs: 10 mM hydroxybenzenesulfonic acid, 10 mM benzoic acid, 3.33 mM trimellitate, 5 mM naphthalenedisulfonic acid, 10 mM hydroxybenzoic acid, 10 mM toluenesulfonic acid, 5 mM chromate, 5 mM phthalic acid, 3.33 mM sulfophthalic acid. All BGEs were buffered with 20 mM DEA to pH 9.2, and 0.5 mM TTAOH added. Separation voltage -25 kV, hydrostatic injection at 10 cm for 5 sec, detection wavelength 254 nm, temperature 25°C. Analyte contained 0.5 mM of each anion.identities as for Figure 4.1. The sample mixture 0.3 mM of each anion*

by peak widths were used to facilitate this comparison. Each peak was normalised to the peak that gave the largest height to width ratio, which in this case was sulfate run with the chromate BGE. Figure *4.6* shows the electropherograms obtained with each probe.It is interesting to note that some of the BGEs could be used over a wider range of analyte mobilities than others. Comparison of hydroxybenzenesulfonic acid (OHBSA), and hydroxybenzoic acid reveals that although these probes both have similar mobilities and are both singly charged, OHBSA displayed superior peak shapes for peaks 9 and 10. Comparison of phthalate and naphthalenedisulfonic acid (NDSA), both of which have two negative charges, shows that NDSA gave superior peaks shapes for the slower moving peaks. Furthermore, the peak symmetry was maintained for more analytes with NDSA than with phthalate. A common feature of OHBSA and NDSA was that they both contain sulfonic acid groups and the results suggest, at least from these two examples, that probes of this type can be used over a wider range of analyte mobilities than those with carboxylic acid groups. In addition, probes with higher charges exhibited superior analyte mobility range, as evident from a comparison of benzoic acid, phthalic acid, and trimellitic acid. Each probe differs by one carboxylic acid group and trimellitic acid clearly shows the best spread of peak shapes over all of the analyte anions. It must be recognised that these trends arise from a very limited number of examples.

Figure *4.7* shows a plot of the height/width ratio for each analyte *versus* the mobility of the analyte for each probe. The best performance was provided by the chromate BGE for the highly mobile anions, and OHBSA for the intermediate and slow moving anions. Due to preferential displacement it is desirable to choose probes that have distinct mobility ranges, rather than probes that give better peak symmetries for the whole range of analytes. Thus, the best multiple probe combination would be a mixture of chromate and OHBSA. There is no advantage in adding a further probe as these two give the performance that is close to optimal for all analyte anions.

Figure *4.8* shows an electropherogram for 15 anions obtained a BGE consisting of an equimolar mixture of chromate and OHBSA. When Fig *4.8* is compared to the electropherograms obtained using the individual probes (Fig *4.6*), superior

performance of the mixed probe BGE is evident. Comparison with the electropherogram obtained with the three-probe BGE (Fig *4.5*) shows that the two-probe BGE has equivalent peak symmetries for the fast moving analytes and superior symmetries for the slower analytes. Moreover, the two-probe BGE also has the advantage of a single system peak. It should also be noted that the system peak in Figure *4.8* was well removed from any analyte peaks so manipulation of the relative concentrations of the two probe co-anions (in order to change the migration time of the system peak) was not necessary in this case.

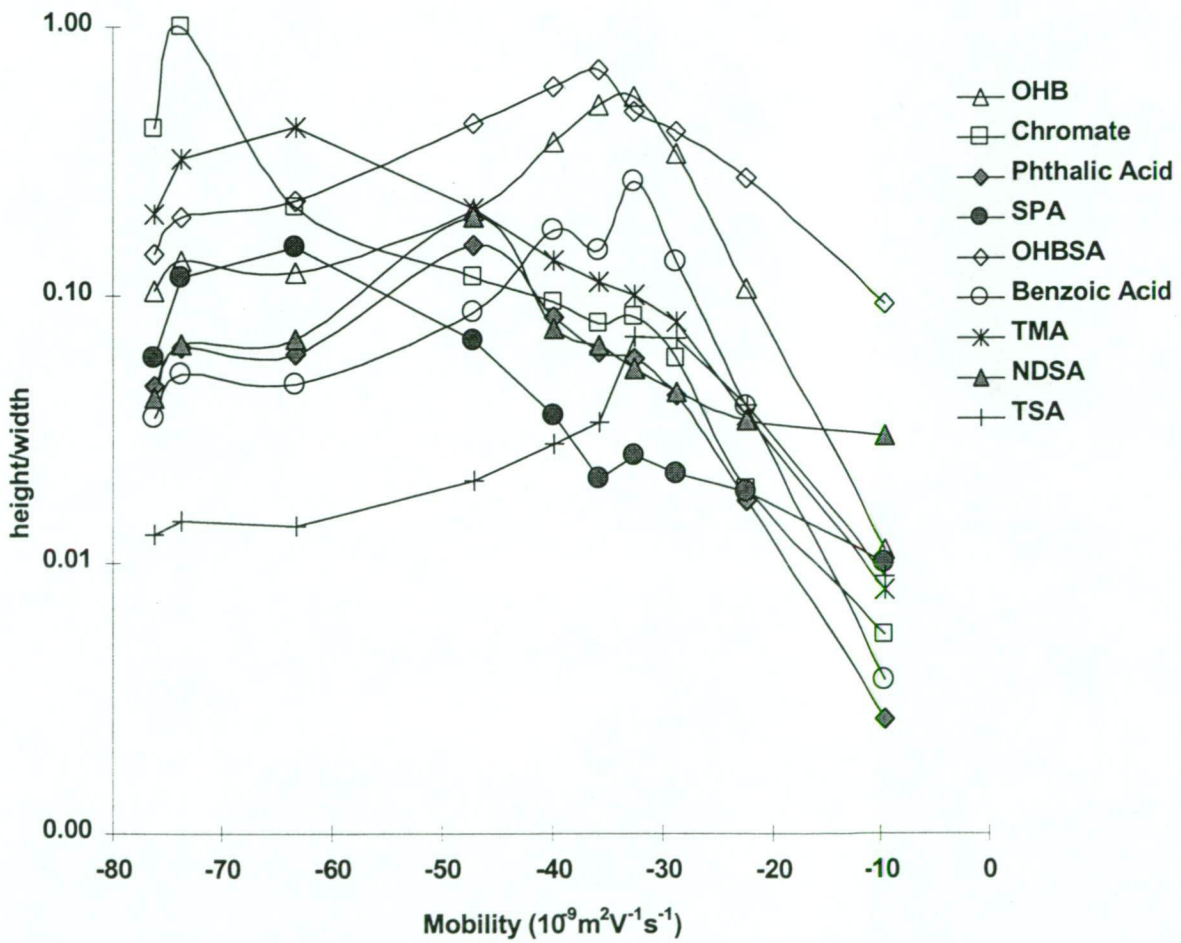


Figure 4.7 *Plot of [normalised height/width] versus analyte mobility for the BGEs shown in Figure 4.6.*

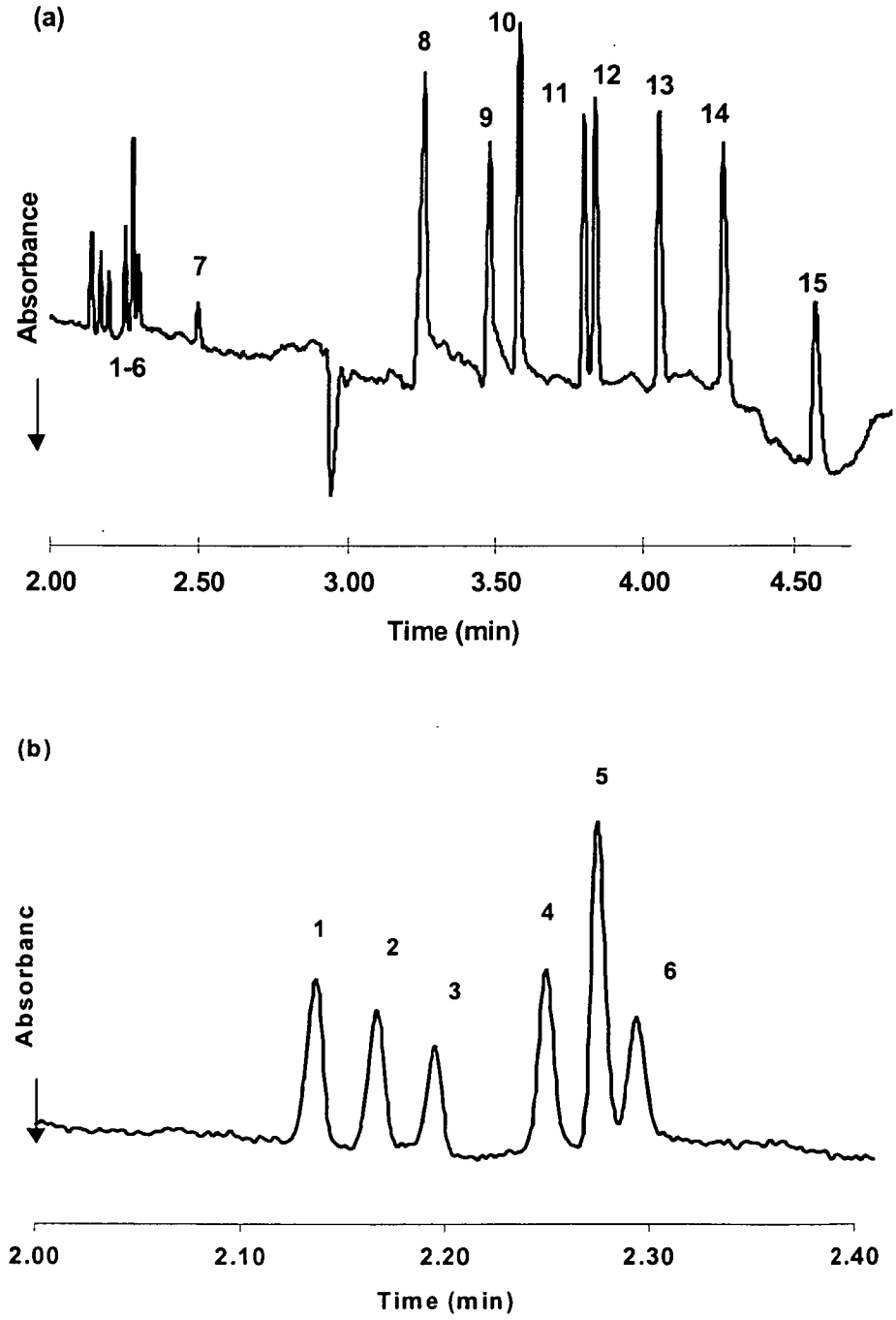


Figure 4.8 *Optimised electropherogram for the separation of 15 analytes using a BGE containing two probes (chromate and hydroxybenzenesulfonate) (a) and zoom view (b). Conditions: BGE: 5.0 mM chromic acid, 5.0 mM hydroxybenzenesulfonic acid, 30 mM DEA, 0.5 mM TTAOH, pH 9.20. Separation voltage -25 kV, hydrostatic injection at 10 cm for 5 sec, detection wavelength 254 nm, temperature 25 °C. Key: 1 = bromide, 2 = chloride, 3 = iodide, 4 = nitrite, 5 = sulfate, 6 = nitrate, 7 = chlorate, 8 = carbonate, 9 = acetate, 10 = ethanesulfonate, 11 = propanoic acid, 12 = propanesulfonate, 13 = butanesulfonate, 14 = pentanesulfonate, 15 = hexanesulfonate. The sample mixture contained 0.2 mM of each anion.*

4.4 Conclusions

The response ratios of analytes using multiple component BGEs containing non-absorbing co anions were found to be dependent on the relative mobilities of the analyte, probe and co-anion. In general, an analyte displaced preferentially the co-anion component of the BGE for which its mobility was closest, and displaced exclusively a co-anion component that had the same mobility.

BGEs with multiple probes were utilised to improve peak shapes by matching the mobilities of the probe co-anions in the BGE with those of as many of the analytes as possible. When n absorbing probe co-anions were added to the BGE, n-1 system peaks were produced. Multiple probe BGEs produced mobility zones between the system peaks in which the co-anions were displaced preferentially by analytes with the closest mobility. Analytes migrating in each zone gave similar peak symmetry to that obtained with the corresponding single probe BGE. The mobility of potentially interfering system peaks for BGEs comprising two probes can be calculated from the mobility of each of the probes and their corresponding concentrations.

A BGE comprising chromate and hydroxybenzenesulfonate as probes was found to give best overall performance in the separation of a mixture of organic and inorganic anions having a wide range of mobilities. This BGE provided improved peak shapes over the electropherogram than when either of the probes was used alone.

4.5 References

[1] G. J. M. Bruin, A. C. Van Asten, X. Xu, and H. Poppe, *J. Chromatogr.*, 608 (1992) 97.

[2] M. W. F. Nielen, *J. Chromatogr.*, 588 (1991) 321.

[3] F. Foret, S. Fanali, L. Ossicini and P. Bocek, *J. Chromatogr.*, 470 (1989) 299.

[4] T. Wang, R. A. Hartwick, *J. Chromatogr.*, 589 (1992) 307.

[5] H. Poppe, *Anal. Chem.* 64 (1992) 1908.

[6] M. Macka, P. R. Haddad, P. Gebauer and P. Bocek, *Electrophoresis*, 18 (1997) 1998.

[7] R. L Williams, and G. Vigh, Poster P123 at Ninth International Symposium on High Performance Capillary Electrophoresis, Anaheim, CA, Jan 1997.

5. Use of dyes as indirect detection probes for the high sensitivity determination of anions.

5.1 Introduction

According to equation (1.3) given in Chapter One (p. 8), increasing the dynamic range, D_r, and minimising detection limits may be achieved by increasing the molar absorbtivity (ε) of the probe [1]. Highly absorbing dyes therefore make ideal candidates as probes in order to maximise indirect detection sensitivity. When such probes are used the concentration of the probe, C_P, is also kept low by the necessity to maintain the background absorbance at such a level to keep the noise reasonable (increasing the background absorbance also increases the noise) and to operate within the linear range of the detector. This is an advantage because low C_P also helps to minimise detection limits.

Several authors have explored various dyes as probes to minimise the detection limits for a variety of ions. Xue *et al* [2] used bromocresol green for the analysis of the pyruvate anion, and malachite green for the detection of potassium, obtaining sub femtomole minimal detectable amounts (MDAs). However, both electrolytes were unbuffered. Mala *et al* [3] also reported sub-femtomole MDAs for cations analysed with the cationic dyes, Chlorophenol Red and Methyl Green. Tris(hydroxymethyl)aminoethane (Tris) was used as a buffering agent, but this species potentially acts as a competing co-cation and thereby decreases sensitivity. They also used Indigo Carmine as the anionic probe for the determination of some inorganic anions, obtaining MDAs at the sub-picomole level. The disappointing MDAs in this latter case were most probably due to the use of acetate as the buffering agent since this would act as a competing co-anion. More recently, Siren *et al* [4] employed nitrosonaphthol dyes in unbuffered electrolytes for the determination of inorganic anions and organic acids, reaching MDAs close to attomole levels. The authors report that attempts to buffer the electrolytes with phosphate at pH 8 were unsuccessful due to deleterious effects on sensitivity.

Thus, buffering of electrolytes for indirect detection provides something of a challenge. In Chapter Four it was shown that detection sensitivity could be severely compromised when co-anions were added to an electrolyte. This is due to competitive displacement between the probe and the added co-anions, resulting in a decrease of the transfer ratio. The extent to which the detection sensitivity is compromised was shown to be dependent upon the relative mobilities of the probe and the co-anion. It was concluded that it is possible to buffer an electrolyte that contained a fast moving probe and a slow moving buffering agent. A further problem with introduction of co-anions is inducement of system peaks, which can interfere with analytes of interest. Alternatively, electrolytes may be buffered for indirect detection of anions by titrating the acid form of a probe with a buffering base to a pH close to the *pKa* of the base (Chapter Three). Another possible alternative for buffering indirect detection electrolytes that has not been explored is the use of ampholytic buffers. When a free ampholyte is dissolved in pure water, the pH of the solution is close to the *pI* of the ampholyte. Under such conditions the ampholyte exists in a zwitterionic form having a net zero charge and therefore does not interfere with indirect detection. An ampholyte will buffer at its *pI* when the *pKa* values of two buffering groups on either side of the pI are close together (within one pH unit). Further, the ampholyte at its *pI* does not contribute to the conductivity of the solution and may be added in sufficiently high quantities to provide good buffering capacity [5].

In this chapter we present separations of C2-C8 alkanesulfonic acids obtained when bromocresol green is used as the probe. The four different buffering protocols mentioned above are investigated to demonstrate the most appropriate approach. These are buffering using a base as the counter-ion (DEA); buffering with an ampholyte (lysine); buffering with a low mobility co-anion (CHES), and buffering with a co-anion that has a mobility close to that of the probe (acetate). The relative merits and disadvantages of each are investigated and the analytical performance of the lysine and DEA buffered electrolytes are compared. Separations of inorganic anions and organic acids are also presented with Indigo–tetrasulfonate as the probe. Two electrolytes were developed, the first buffered with the base 1,3-bis[tris(hydroxymethyl)-methylamino]-propane (Bis Tris), and the second with the

ampholyte, glutamic acid. Analytical performance of each of the electrolytes is also presented. Finally, the further improvement of detection limits is demonstrated when the effective pathlength of the detection cell is increased with a Z cell.

5.2 Experimental

The general experimental is given in Chapter Two. Detailed conditions are included in each of the figure captions.

5.2.1 Preparation of Lysine

The *pI* of lysine is 9.7, but dissolution of free lysine in water resulted in a solution having a pH of 10.2. This pH was too high for indirect detection methods since lysine would carry a net negative charge at this pH and therefore would act as a competing co-anion. Three different batches of lysine obtained from Fluka, Sigma and Aldrich showed similar behaviour and attempts at purifying lysine by recrystallisation procedures proved unsuccessful.

Cationic impurities were removed by utilising a dialysis apparatus which consisted of a 160 cm x 0.6 mm ID Du Pont (Delaware, USA) Nafion cation-exchange hollow fibre packed with polystyrenedivinylbenzene beads, coiled on a holder made of glass rods and placed in a housing tube made from clear acrylic material (20 cm x 25 mM ID). [6]. The device contained a solution of 0.1 *M* camphorsulfonic acid solution and 25g of Biorad Dowex 50w x16 cation exchange resin (H^+ form) which surrounded the hollow-fibre and acted as the hydrogen ion donating medium. When a 0.1 *M* solution of lysine was pumped through the fibre at 0.1 ml per min, the resulting effluent had a pH of 9.7.

5.2.2 Preparation of Acid Form of ITS

5g of Bio Rad Dowex 50 w x16 cation-exchange resin was dry packed into a disposable 10 ml syringe. The packed syringe was washed with 200 ml of 0.1 *M* hydrochloric acid. A vacuum was applied to provide a flow rate of approximately 30 ml/min. The syringe was then washed with 200 ml of Milli Q water, or until the pH of the effluent was close to neutral. 50 ml of a 10 m*M* solution of the potassium salt

form of ITS was then passed through the packed syringe at a flow rate of approximately 10 ml/min. The first 10 ml was discarded and the rest of the effluent collected. The collected fraction was used for subsequent preparation of electrolytes buffered with buffering bases.

5.2.3 Calculation of Minimal Detectable Amounts (MDAs)

The MDAs were calculated at a signal to noise ratio of two. The noise was calculated as the root mean square of a 1 min segment of stable baseline using Turbochrom software.

The amount of solute injected into the capillary was calculated from the Poiseuille equation [7]:

$$w = \frac{\Delta P \Pi r^4 C t_i}{8 \eta L} \qquad (5.1)$$

where w = amount of solute injected (mole), ΔP = pressure difference (Pa), r = radius of inner diameter of capillary (m), C =concentration of injected solute (mol/l), t_i = time of injection (s), η = viscosity (Pa.s), L = total length of capillary (m).

If sample injection is accomplished by gravity the pressure difference (ΔP) is given by the hydrostatic pressure defined as:

$$\Delta P = \rho g \Delta h \qquad (5.2)$$

where ρ = density of the electrolyte (Kgm^{-3}), g = gravitational acceleration (9.8 $N.kg^{-1}$), Δh = height difference between liquid levels of sample and buffer vials (m).

5.3 Results and Discussion

5.3.1 Selection of Dyes

For high sensitivity indirect detection, the UV absorbing component of the electrolyte (probe) must have a high molar absorptivity at the wavelength chosen and the molar concentration of the probe should be kept low. The charge of the probe must also be stable at the pH of the electrolyte. Two candidates were chosen to fulfil these requirements: bromocresol green (BCG) and indigo-tetrasulfonate (ITS). Data relevant to these two dyes is given in Table 5.1, from which it can be seen that BCG has intermediate mobility and was therefore chosen for the determination of intermediate to low mobility anions. Further, BCG (p*Ka*=4.9 [8]) has two negative charges and its molar absorbtivity is constant at pH 7 or higher, making it suitable for electrolytes buffered at pH>7.

ITS has a high to intermediate mobility and was therefore chosen as a candidate for the determination of anions of similar mobility. ITS has four negative charges and its molar absorptivity is constant over a very wide pH range, making it suitable for electrolytes buffered at most values of pH.

5.3.2 Buffering Protocols

BCG was chosen as the probe for the investigation of the four buffering protocols detailed in the introduction. Table 5.2 presents the composition of each of the electrolytes. In all cases the probe concentration was kept at 0.5 m*M*. Electrolyte 1 consisted of the free acid of BCG titrated with DEA to the *pKa* of DEA. This electrolyte provided a moderately buffered two component electrolyte. Electrolyte 2 was buffered with the ampholyte, lysine. Electrolyte 3 was buffered with the low mobility buffer CHES. Electrolyte 4 was buffered with a DEA-acetate buffer.

A series of 7 sulfonic acids (C2-C8) was used as analytes that encompassed the useful mobility range of BCG (10 to 40 $10^{-9}m^2V^{-1}s^{-1}$). Figure *5.1* shows the electropherograms obtained for the solutes with each of the above electrolytes.

Table 5.1 Selected characteristics of dyes used as probes

Dye	Experimental Wavelength (nm)	Molar Absorptivity [a] ($x10^3$ $mol^{-1}l$ cm^{-1})	Mobility [b] ($10^{-9}m^2V^{-1}s^{-1}$)
BCG	618	45.0	-36.3
ITS	314	32.1	-70.7

[a] Calculated from spectra measured of 0.005m*M* BCG buffered to pH 9.2 with DEA, and 0.05 m*M* of ITS buffered to pH 6.8 with bis tris.

[b] Mobility of each dye was experimentally determined. Electrolyte: 1.0m*M* phthalic acid buffered to pH 9.2 with DEA. Conditions: 30kV, 618 nm, 0.6s vacuum inj @ 5" Hg.

Table 5.2 Electrolyte compositions for buffer investigation

	Probe	Buffer	pH
Electrolyte 1	0.5m*M* BCG	2.0 m*M* DEA	9.20
Electrolyte 2	0.5m*M* BCG	10 m*M* Lysine	9.70
Electrolyte 3	0.5m*M* BCG	2.0 m*M* CHES	9.55
Electrolyte 4	0.5m*M* BCG	2.0 m*M* Acetate, 4.0 m*M* DEA	9.20

In all cases the analytes were migrating in a direction opposite to the flow of EOF, but the EOF had sufficient electrophoretic velocity to sweep the analytes in the same direction as the EOF. Consequently the anion of lowest mobility (octanesulfonate) migrated from the capillary first, and the most mobile anion (ethanesulfonate) migrated last. Detection was performed at the cathodic end of the capillary. An EOF modifier such as tetradecyltrimethylammonium bromide (TTAB) could not be added to reverse the EOF because an insoluble precipitate was produced between the modifier and the dye.

The DEA buffered electrolyte provided baseline separation of all of the analytes, as well as very good sensitivity. The lysine buffered electrolyte also provided baseline

resolution. For the CHES buffered electrolyte, peaks 1 and 2 were diminished in area, peak 3 completely disappeared, a system peak was induced that completely swamped peak 4, peaks 5 and 6 were relatively unaffected, and peak 7 was halved in area. These effects were caused by competitive displacement of the buffer anion at the expense of the probe. Consequently, CHES is unsuitable for buffering the moderately mobile BCG. The acetate-DEA buffer exhibited the same undesirable

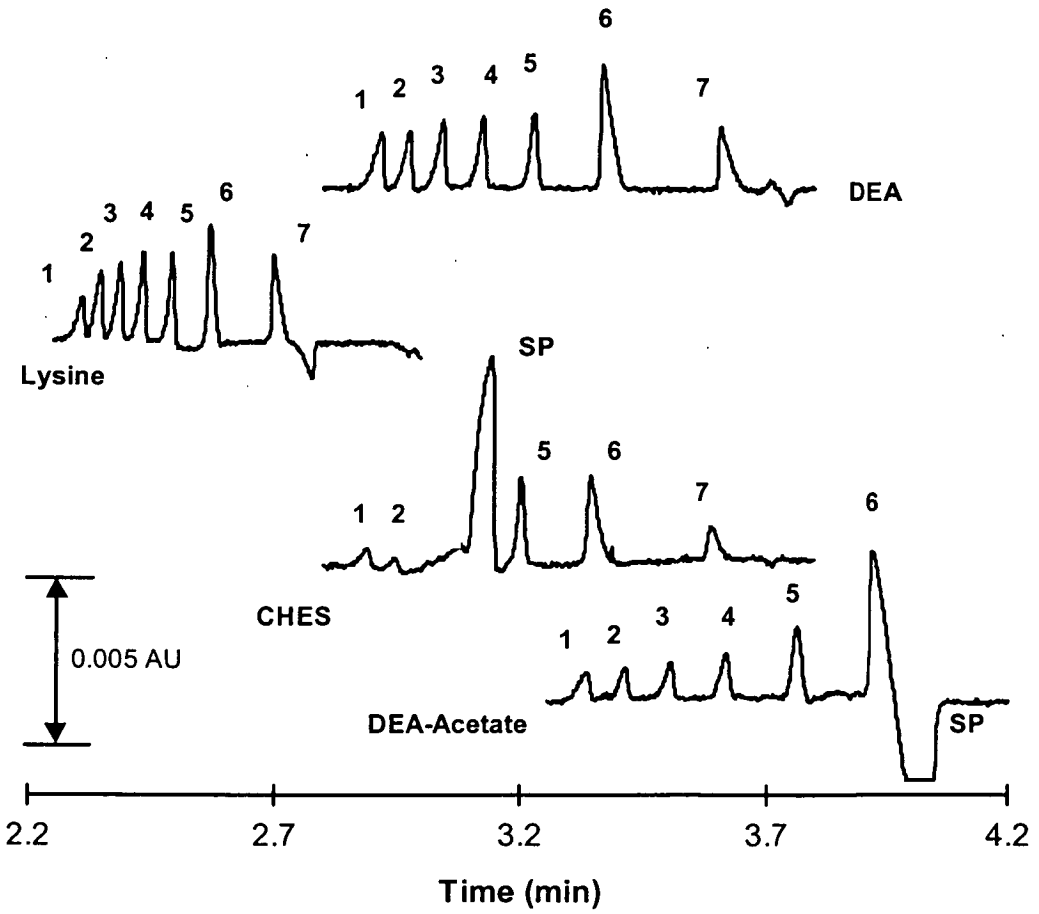


Figure 5.1 *Electropherograms obtained with different buffering protocols.. Conditions: electrolyte given in Table 5.2, separation voltage 30 kV, 2 s pressure injection at 5" Hg, detection wavelength 618 nm, temperature 30°C. 10 μM of each anion. Key: 1 = octanesulfonate, 2 = heptanesulfonate,3 = hexanesulfonate, 4 = pentanesulfonate, 5 = butanesulfonate, 6 = propanesulfonate, 7 = ethanesulfonate, SP = system peak.*

effects for similar reasons. This behaviour explains the observation [3] that relatively poor detection sensitivities for the detection of anions using the dye chlorophenol red as the probe in a buffer consisting of 1 m*M* Tris adjusted with acetic acid to pH 6.0-7.2. The concentration of acetate in this electrolyte was approximately 1 m*M*, which was two to ten times greater than the concentration of chlorophenol red (0.1 – 0.5 m*M*). The introduced co-anion acetate reduced the transfer ratio and therefore the detection sensitivity.

5.3.3 Analytical Performance Parameters with BCG as Probe

Maximum transfer ratios (and hence maximum theoretical sensitivity) as well as good reproducibility can be achieved when the acid form of the probe is titrated with a buffering base (Chapter Three). Accordingly, a comparison of sensitivity, calibration linearity and reproducibility obtained with BCG as the probe buffered either with DEA or lysine (electrolytes 1 and 2, Table 5.2) was performed. The results are given in Table 5.3.

Calibration curves of corrected area peak area *versus* molar concentration of the 7 sulfonic acids used earlier were constructed from duplicate injections (0.6s @ 5" Hg) of 2-50 μ*M* of each sulfonic acid for both electrolytes, without replenishing the electrolyte reservoirs between injections. The correction made to peak area was to account for the different migration velocities of the sample bands and was achieved by dividing the peak area of each solute by its migration time. Comparisons of the resulting correlation coefficients for the calibration plots showed no significant difference between the two electrolytes, with linearity being maintained for each solute up to at least 50 μm. The migration time reproducibility was calculated from 10 replicate injections for each analyte and in all cases the lysine buffered electrolyte gave superior migration time reproducibility compared to the DEA buffered electrolyte. This was due to the higher buffering capacity of the lysine electrolyte. The DEA electrolyte was moderately buffered but its buffering capacity was limited to the concentration of the probe as a result of the titration method used to prepare the electrolyte. The probe concentration could not be further increased to increase the

Table 5.3 **Analytical performance parameters of BCG electrolyte buffered with lysine or DEA**

Solute	Buffer	pH	Correlation Coefficient (r^2)	Migration Time Precision (% RSD)	LOD	
					MDA (mole)	Conc. (μM)
bromide	bis tris	6.80	0.9950	1.4	$2x10^{-17}$	2
chloride	bis tris	6.80	0.9878	1.4	$2x10^{-17}$	2
sulfate	bis tris	6.80	0.9989	1.7	$3x10^{-18}$	0.3
thiocyanate	bis tris	6.80	0.9994	1.2	$5x10^{-18}$	0.4
chlorate	bis tris	6.80	0.9996	1.1	$5x10^{-18}$	0.4
malonate	bis tris	6.80	0.9998	0.7	$2x10^{-18}$	0.2
tartrate	bis tris	6.80	0.9996	0.3	$1x10^{-18}$	0.1
bromate	bis tris	6.80	0.9988	0.3	$3x10^{-18}$	0.3
formate	bis tris	6.80	0.9792	0.2	$6x10^{-18}$	0.5
succinate	bis tris	6.80	0.9999	0.1	$2x10^{-18}$	0.2
phthalate	bis tris	6.80	0.9999	0.2	$2x10^{-18}$	0.2
iodate	bis tris	6.80	0.9993	0.6	$5x10^{-18}$	0.4
phosphate	bis tris	6.80	0.9976	0.7	$7x10^{-18}$	0.6
sulfate	glutamic acid	3.22	0.9987	0.8	$1x10^{-17}$	0.9
nitrate	glutamic acid	3.22	0.9994	0.4	$1x10^{-17}$	1
perchlorate	glutamic acid	3.22	0.9989	0.2	$1x10^{-17}$	1
chlorate	glutamic acid	3.22	0.9996	0.3	$1x10^{-17}$	0.9
bromate	glutamic acid	3.22	0.9971	0.6	$8x10^{-18}$	0.7

buffering capacity because the background absorbance became unacceptably high, increasing the baseline noise and moving the detector out of its linear response range.

The slopes of the calibration curves were quite different for the two electrolytes, with the lysine buffered electrolyte giving responses that were approximately 70% of those obtained with the DEA buffered electrolyte. The stock dialysed lysine solution was analysed using the buffered chromate electrolyte detailed in Chapter Six, and found to contain approximately 2 m*M* carbonate that presumably had been absorbed from the atmosphere during the dialytic clean-up step [9]. This resulted in an electrolyte containing 0.2 m*M* carbonate, which acted as a competing co-anion and thereby reduced the detection sensitivity . Despite this drawback, the results showed that ampholytes could be used successfully to buffer electrolytes used for indirect detection.

The LODs for each of the sulfonic acids are reported in Table 5.3 as MDAs, and the corresponding solution concentrations in μ*M*. Unlike LODs expressed as solution concentrations, MDAs are independent of the injection volume and provide a more reasonable comparison of sensitivities achieved between different studies. The MDAs were calculated using eqn. (5.1) and based on 0.6 s pressure injection at 5" Hg, and a 70 cm x 75 μm capillary. The MDAs for the sulfonic acids range from 1-3 $x10^{-18}$ mole for the DEA buffered electrolyte, corresponding to solution concentrations of around 0.2 μ*M*. Shamsi and Danielson [10] have reported detection limits for C4-C14 alkanesulfonates of 0.5-2.0 mg/l using naphthalenemonosulfonic acid as the probe and detection at 214 nm. These convert to MDAs (calculation based upon 12s pressure injection at 5" Hg, and 50 μ*M* capillary) of approximately 1 $x10^{-16}$ mole, or about 1.5 orders of magnitude higher than the MDAs obtained with BCG.

The analysis of the sulfonic acids using BCG buffered with DEA is believed to be the most sensitive yet reported for C2 to C8 alkane sulfonic acids using capillary electrophoresis and indirect photometric detection. Indeed, the LODs (10^{-6} *M* injected sample concentration) are at least an order of magnitude lower than the

reported generalised detection limit for the technique of indirect detection (10^{-4} to 10^{-5} *M*) [11].

5.3.4 Use of ITS as the Probe

The analysis of higher mobility solutes cannot be achieved in the same way as used for the low mobility analytes because the EOF does not have sufficient velocity to carry the high mobility analytes to the detector. Additionally, BCG does not have a suitable mobility to provide adequate peak shapes for these anions [12]. Accordingly, the higher mobility probe ITS was investigated, with the separation polarity being reversed. In this case, suppression of the EOF was desirable but since this could not be achieved by the usual method of addition of a surfactant (due to precipitate formation), it was necessary to develop alternative electrolytes that suppressed the EOF. Two approaches were used to achieve this. The first was titrating the acid form of ITS with the buffering base, Bis Tris which has been used earlier in Chapter Three in the determination of high mobility anions without the need for EOF flow reversal. The second approach for the analysis of fast anions was to use the low pH ampholytic buffer, glutamic acid.

The first approach is illustrated in Figure *5.2(a)* which shows an electropherogram of 14 anions obtained with ITS buffered with Bis Tris, whilst Fig *5.2(b)* shows the same anions present at concentrations near their detection limit. Table 5.4 gives the analytical performance parameters obtained with ITS buffered with Bis Tris. Calibration curves constructed for each of the analytes showed good linearity with the exception of citrate, which adsorbed onto the capillary wall and was not detected at concentrations less than 20 μ*M*. The linearity limits for bromide and chloride were approximately 100 μ*M*, and for the rest of the analytes were approximately 50 μ*M*. The migration time RSDs range from 0.1-1.4%, with the better RSDs being associated with the most symmetrical peaks. The MDAs ranged from $1x10^{-18}$ to $2x10^{-17}$ mole, with the lowest values being obtained for the most symmetrical peaks and for analytes of higher charge. These values compared favourably with those obtained for the alkanesulfonic acids using BCG, and are again at least an order of magnitude lower than the general detection limit for indirect detection reported in ref [11].

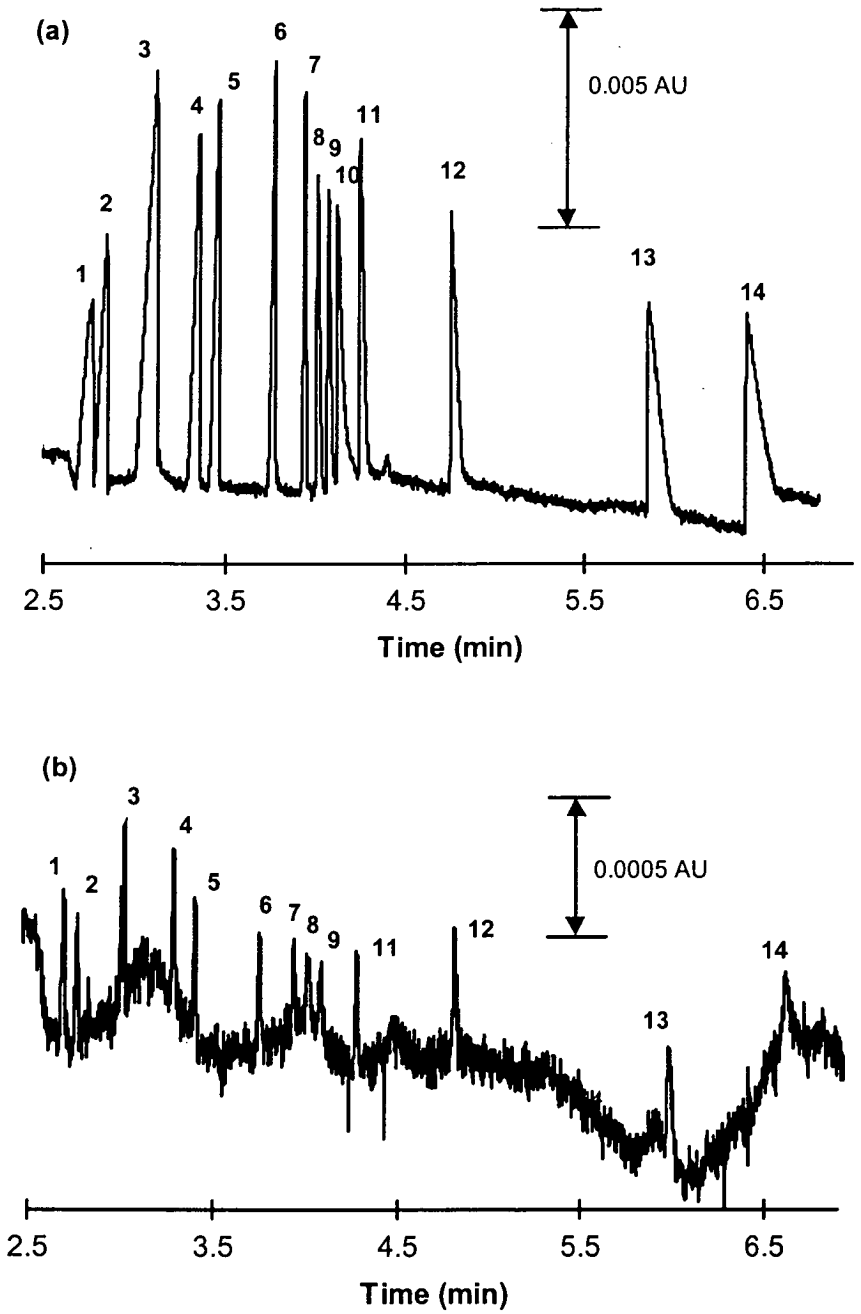


Figure 5.2 *Electropherogram of 14 anions obtained from the high concentration standard (a) and near their detection limits (b) with ITS as probe. Conditions: electrolyte 0.5 mM ITS, 2.67 mM Bis Tris, pH=6.8, separation voltage -30 kV, 0.6 s pressure injection @ 5" Hg, detection wavelength 314 nm, temperature 30°C. Key: 1 = bromide (a) 200 μM, (b) 10 μM; 2 = chloride (a) 200 μM, (b) 10 μM; 3 = sulfate (a) 80 μM, (b) 2 μM; 4 = thiocyanate (a) 80 μM, (b) 2 μM; 5 = chlorate (a) 80 μM, (b) 2 μM; 6 = malonate (a) 40 μM, (b) 1 μM; 7 = tartrate (a) 40 μM, (b) 0.5 μM; 8 = bromate (a) 40 μM, (b) 1 μM; 9 = formate (a) 40 μM, (b) 1 μM; 10 = citrate (a) 30 μM, (b) –; 11 = succinate (a) 30 μM, (b) 0.8 μM; 12 = phthalate (a) 30 μM, (b) 0.8 μM; 13 = iodate (a) 60 μM, (b) 1.5 μM; 14= phosphate 60 μM, (b) 1.5 μM.*

Table 5.4 Analytical performance parameters for ITS used as probe

olute	Correlation Coefficient (r^2)		Migration Time Precision (% RSD)		Calibration Slope ($\mu Vs/\mu M$)		LOD			
							Lysine		DEA	
	Lysine	DEA	Lysine	DEA	Lysine	DEA	MDA (mole)	Conc. (μM)	MDA (mole)	Conc. (μM)
Octane SA	0.9977	0.9985	1.4	1.7	31	70	$3x10^{-17}$	2	$2x10^{-18}$	0.2
Heptane SA	0.9973	0.9992	1.5	1.6	45	64	$2x10^{-17}$	1	$2x10^{-18}$	0.2
Hexane SA	0.9974	0.9975	1.5	1.7	47	69	$2x10^{-17}$	1	$2x10^{-18}$	0.2
Pentane SA	0.9984	0.9991	1.5	1.8	49	68	$2x10^{-17}$	1	$3x10^{-18}$	0.3
Butane SA	0.9944	0.9986	1.5	1.8	50	65	$2x10^{-17}$	2	$2x10^{-18}$	0.2
Propane SA	0.9872	0.9967	1.2	2.0	51	83	$1x10^{-17}$	1	$1x10^{-18}$	0.1
Ethane SA	0.9965	0.9995	1.3	2.2	54	71	$1x10^{-17}$	1	$2x10^{-18}$	0.2

Use of the second of the above mentioned approaches was straightforward since glutamic acid produced a solution of the expected pH (i.e. at the *pI* of 3.22). This allowed the indirect detection buffer to be prepared by dissolving the sodium salt of the probe in an appropriate concentration of the ampholyte, without the need to convert the probe into its acid form as was required when buffered with Bis Tris. The EOF was suppressed sufficiently at pH 3.22 to allow migration of fast anions in a counter EOF mode with detection at the anode. However this method was limited to the determination of relatively strong acid anions due to the low pH of the electrolyte. Figure *5.3(a)* shows the separation of 5 inorganic anions obtained with 0.2 m*M* ITS buffered to pH 3.22 with 10 m*M* glutamic acid. Figure *5.3(b)* demonstrates the improvement in random baseline fluctuations (chemical noise) by

addition of 0.1% carbowax 20 M to the electrolyte. The random baseline fluctuations were thought to occur due to hydrophobic interactions between the dye and the capillary wall. Carbowax 20 M was added to the electrolyte to make the walls of capillary more hydrophilic, thereby preventing the adsorption of the dye.

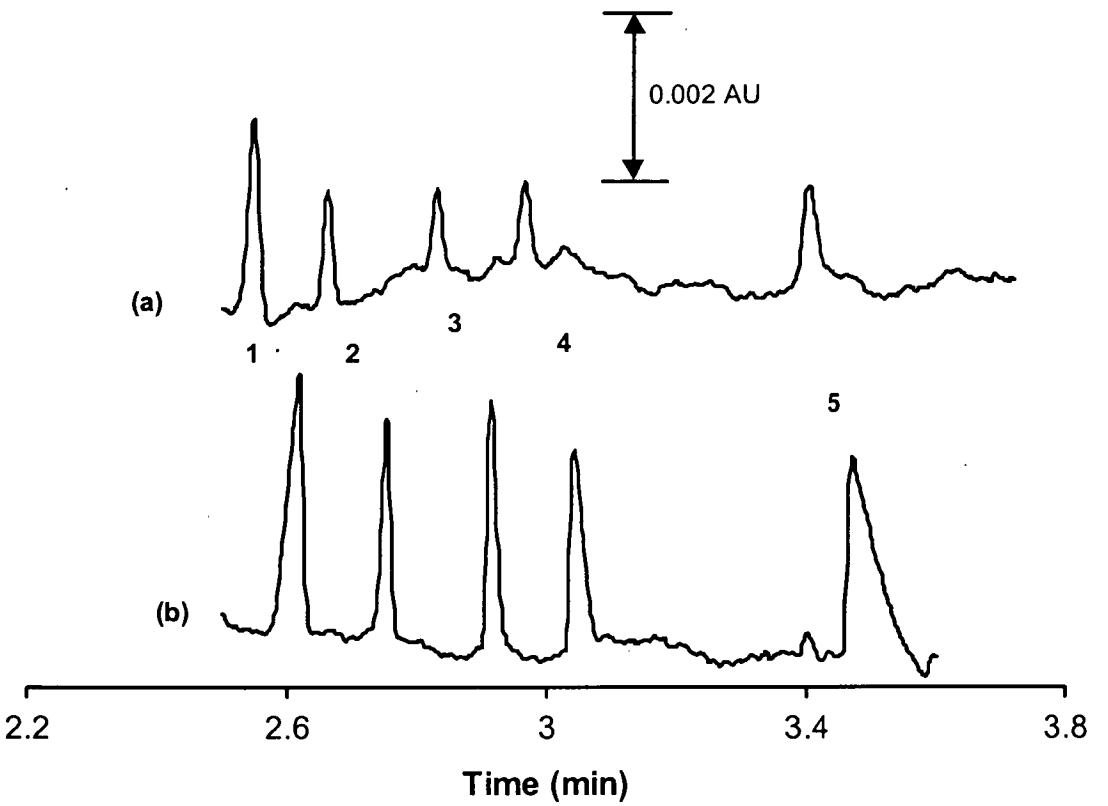


Figure 5.3 *Electropherogram of 5 anions obtained with ITS as probe (a), and improvement in baseline noise with carbowax 20 M added to the electrolyte (b). Conditions: electrolyte (a) 200 μM ITS, 10mM glutamic acid, pH = 3.22. , (b) 200 μM ITS, 10 mM glutamic acid, 0.1 % carbowax, pH = 3.22, separation voltage -30 kV, 0.6 s pressure injection @ 5" Hg, detection wavelength 314 nm, temperature 30°C, sample (a) 10 μM (b) 20 μM of each anion. Key: 1 = sulfate, 2 = nitrate, 3 = perchlorate, 4 = chlorate, 5 = bromate.*

Analytical performance parameters were determined as before and are reported in Table 5.4. Calibration linearities were excellent for all solutes up to 100 μM, and the migration time reproducibilities are all less than 1%. Detection limits were at the 1 x 10^{-17} mole level, which translates into solution concentrations around 1 μM. To illustrate the improvement in detection limits obtained using this approach, electropherograms of the same anions obtained with ITS and the commonly used chromate electrolyte are shown in Figure *5.4*. The same experimental conditions were used in each case with the exception of wavelength, which was set to the respective absorption maximum for each probe. Perchlorate (peak 3) does not appear in the chromate trace because it co-migrates with chlorate. Noise levels in each electropherogram were approximately the same, but the peak heights were ten times greater for ITS.

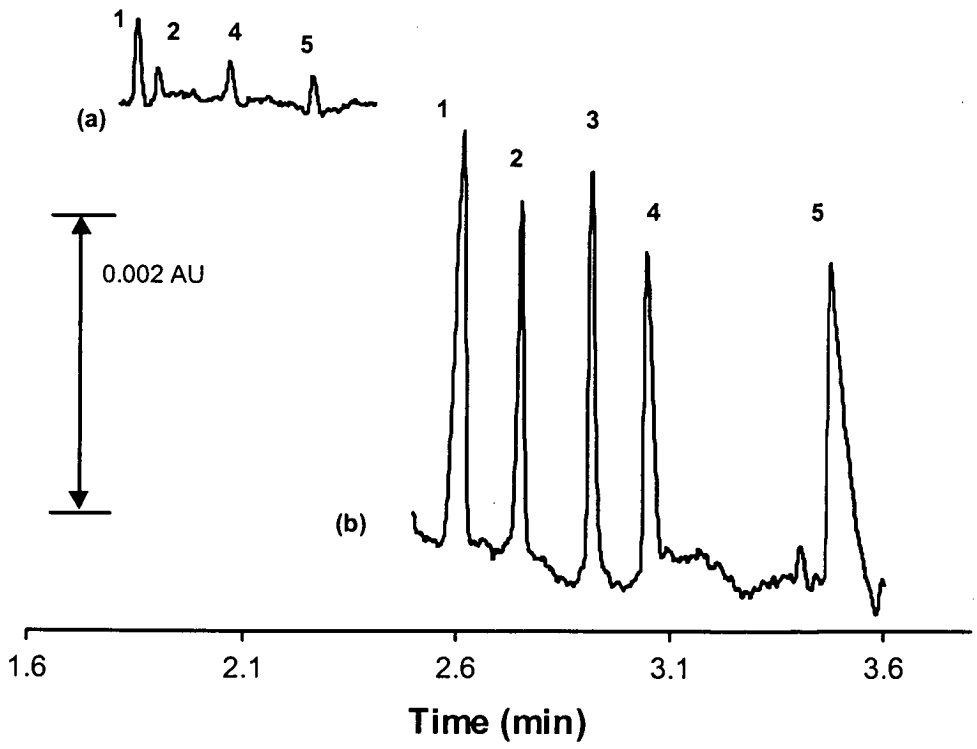


Figure 5.4 *Comparison of chromate (a) and ITS (b) as probes. Conditions: electrolyte (a) 5 mM chromic trioxide, 20 mM DEA, 0.5 mM TTAB, pH = 9.1, (b) 200 M ITS, 10 mM glutamic acid, 0.1 % carbowax, pH = 3.22, separation voltage -30 kV, 0.6 s pressure injection at 5" Hg, detection wavelength 314 nm, temperature 30°C, sample 20 M of each anion. Key: 1 = sulfate, 2 = nitrate, 3 = perchlorate, 4 = chlorate, 5 = bromate.*

5.3.5 Z Cell

The sensitivity of direct detection has been shown to improve by between 5 and twenty times by bending a capillary and illuminating through the bend [10]. Such an arrangement is known as a Z cell. The increase in sensitivity is due to an increase in optical pathlength by about 40 fold. So far, Z cells have only been applied to direct detection [13,14].

Figure *5.5* shows electropherograms for a selection of analytes of intermediate to high mobility obtained with ITS as the probe using either the normal cell arrangement (optical pathlength 75 μm) or the Z cell (optical pathlength 3 m*M*). The detection

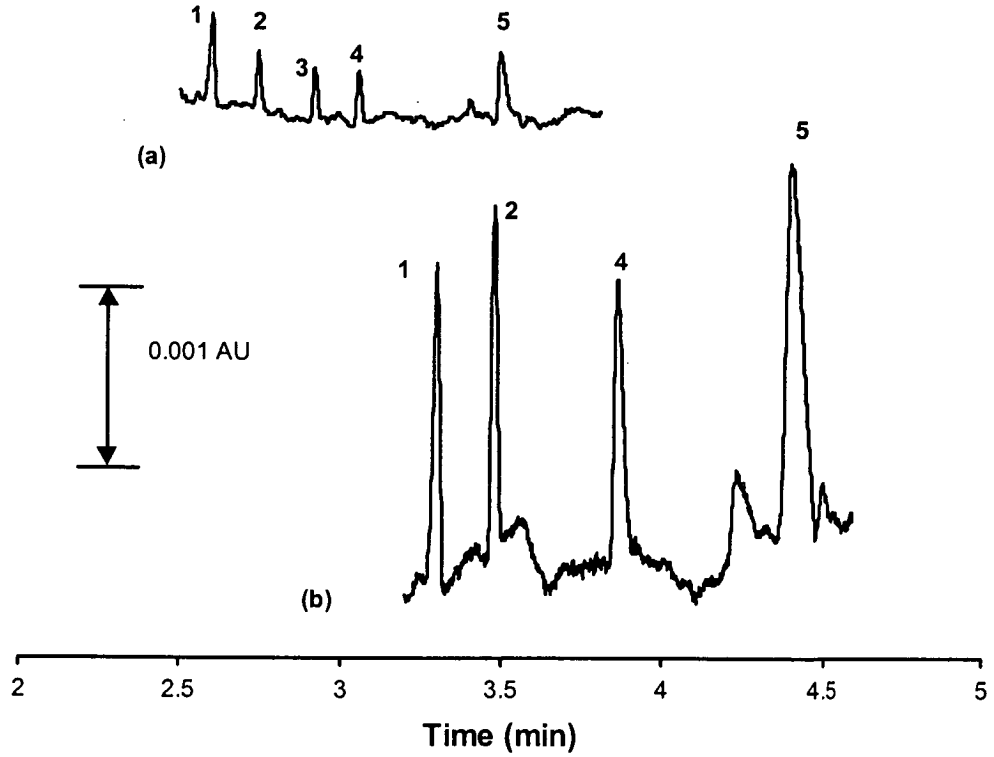


Figure 5.5 *Comparison of normal cell (a) and Z cell (b).*
Conditions: electrolyte (a) 200 μM ITS, 10 mM ITS, 0.1 % carbowax, pH = 3.22, (b) 50 μM ITS 2.5 mM glutamic acid, 0.1 % carbowax, pH = 3.22, separation voltage -30 kV, 0.6 s pressure injection at 5" Hg, detection wavelength 314 nm, temperature 30°C, sample 5 μM of each anion.
Key: 1 = sulfate, 2 = nitrate, 3 = perchlorate, 4 = chlorate, 5 = bromate.

sensitivity was increased by about 3 times, giving MDAs of the order of 3 $x10^{-18}$ mole for a singly charged solute. This is approximately in line with the detection limit achieved with indirect fluorescence detection [11]. Some practical problems must be resolved for the Z cell separation to work successfully. These mainly concern the increase in background absorbance (by a factor of 13) due to the increase in optical pathlength. This necessitated a reduction in the probe concentration to keep the detector noise to a reasonable level and to maintain a linear response. The concentration of ITS for this analysis was 50 μM, and the corresponding background absorbance was 0.127. The only viable means to buffer such an electrolyte was to use an ampholyte. Due to the low probe concentration, titrating with a buffering base would result in a very weakly buffered electrolyte. Buffering with agents that introduce co-anions would not be suitable for reasons discussed above.

5.4 Conclusions

Indirect photometric detection sensitivity in CZE of low molecular weight anionic analytes was improved by at least an order of magnitude when highly absorbing dyes were used as probes. Buffering these types of electrolytes could be achieved with an ampholytic buffer or with a buffering base. Buffers that introduced co-anions were unsuitable because the detection sensitivity was compromised and potentially interfering system peaks were induced.

Bromocresol green was found to be a suitable probe for the analysis of C2-C8 alkanesulfonic acids and when buffered with DEA gave detection limits at attomole levels. The detection limits were not as good when bromocresol green was buffered with the ampholyte lysine. This was caused by absorption of carbon dioxide from the air, resulting in an electrolyte that contained competing co-anions that reduced the transfer ratio. Nevertheless, the results have shown that ampholytic buffers were effective for buffering indirect detection electrolytes.

Indigo tetrasulfonate proved to be a suitable probe for the analysis of small inorganic anions and organic acids when buffered with the base Bis Tris. The detection limits achieved were again at attomole levels, with migration time reproducibilities generally less than 1% RSD. Indigo tetrasulfonate was also a suitable probe for the

analysis of strong acid inorganic anions when buffered at low pH with the ampholyte glutamic acid. This electrolyte gave ten times higher sensitivities than the commonly used chromate electrolyte and migration time reproducibilities were also less than 1% RSD.

Finally the detection sensitivity could be further increased by three times when a Z cell was employed as the detection cell. However, when such a cell was used it was necessary to reduce the probe concentration to 50 μM. The only viable means to buffer this electrolyte was to use an ampholyte.

5.5 References

[1] F. Foret, S. Fanali, L. Ossicini and P. Bocek, *J. Chromatogr.*, 470 (1989) 299.

[2] Y. J. Xue and E. S. Yeung, *Anal. Chem.*, 65 (1993) 2923.

[3] Z. Mala, R. Vespalec and P. Bocek, *Electrophoresis*, 15 (1994) 1526.

[4] H. Siren, A. Maattanen and M. L. Riekkola, *J. Chromatogr*, 767 (1997) 293.

[5] S. Hjerten, L. Valtcheva, K. Elenbring and J. L. Liao, *Electrophoresis*, 16 (1995) 584.

[6] S. Laksana, PhD Thesis, University of Tasmania, (1993) 65.

[7] R. Kuhn and S. Hoffstetter Kuhn, Capillary Electrophoresis: Principles and Practice, Springer Laboratory (1993).

[8] CRC Handbook of Chemistry and Physics: CRC Press, Boca Raton, 1994.

[9] M. Macka, P. R. Haddad, P. Gebauer and P. Bocek, *Electrophoresis*, 18 (1997) 1998.

[10] S. A. Shamsi and N. D. Danielson, *Anal. Chem.*, 66 (1994) 3757.

[11] M. Albin, P. D. Grossman and S. E. Moring, *Anal. Chem.*, 65 (1993) 489A.

[12] F. E. P. Mlkkers, F. M. Everaerts and T. P. E. M. Verheggen, *J. Chromatogr.*, (1979).

[13] S. E. Moring, R. T. Reel and R. E. J. Van Soest, *Anal. Chem.*, 65 (1993) 3454.

[14] J. P. Chervet, R. E. J. Van Soest and M. Ursem, *J. Chromatogr.*, 543 (1991) 439.

6. Buffering Indirect Absorbance Electrolytes

6.1 Counter-Cation Buffered Chromate Electrolytes for Separation and Indirect Absorbance Detection of Inorganic Anions

6.1.1 Introduction

The most commonly used background electrolytes (BGEs) for indirect absorbance detection of inorganic anions and low molecular weight carboxylic acids contain an absorbing anionic probe ion (e.g. chromate), usually at a pH where it is fully dissociated. The cations in such electrolytes are usually non-buffering, e.g. sodium, and consequently the BGE as whole is unbuffered, or at least its buffering capacity is very low. A typical example is sodium chromate at a pH of around 8, introduced in 1990 by Jones and Jandik [1]. This has become one of the most frequently used electrolyte systems for determination of low molecular weight anions by CE and numerous studies have been published detailing the optimisation of separations and detection sensitivities [2-11].

A practical drawback of using sodium chromate as the BGE is that it lacks buffering capacity. Buffering of the BGE in CE is essential to stabilise electroosmotic flow (EOF) and migration time of the analytes [12]. Recently a study showing large changes of pH (several units) due to electrolysis in unbuffered BGEs [13, 14] has been presented. Despite its popularity, little work has been presented on approaches for buffering of BGEs for indirect detection of anions, where a UV-absorbing co-ion is present in the BGE and detection is accomplished as a result of displacement of this probe ion by the analyte ions. Buffers that introduce additional co-anions into the BGE system are undesirable as they cause interfering system peaks [15, 16], and can reduce detection sensitivity due to competitive displacement of the buffer co-ion rather than the probe co-ion [17]. The buffering of pH of BGEs for indirect detection of anions without introduction of co-anions can be achieved using a suitable buffering base as a counter-ion of the probe. Surprisingly little work has been done using this approach. Francois *et al* [2] added triethanolamine (TEA) to a potassium

dichromate BGE to increase the buffering capacity, but only a limited buffering capacity was achieved. Analytical performance characteristics were not reported.

In the following section, an evaluation of two buffered chromate electrolytes is presented. Dissolved chromic trioxide was titrated with the buffering base tris(hydroxymethyl)aminoethane (Tris) and with diethanolamine (DEA) to a pH close to the *pKa* of the base. These electrolytes have a good buffering capacity and provide improved analytical performance characteristics such as migration time repeatability and peak area repeatability. It was the aim of this study to investigate the suitability of the buffered BGE and to provide a comparison of analytical performances for the buffered and unbuffered chromate electrolytes.

6.1.2 Experimental

The general experimental is given in Chapter Two. Detailed conditions are included in each of the figure captions.

6.1.2.1 Procedures

The unbuffered chromate electrolyte was prepared by titration of chromic trioxide with NaOH to pH 8.5 to a final concentration of 5m*M* chromate. 0.5 m*M* TTAB was added to reverse the EOF so that separations could be performed in the co-electroosmotic mode. The buffered chromate electrolytes were prepared by titration of chromic trioxide with Tris (*pKa*=8.5) or DEA (*pKa*=9.2) to their respective *pKa* values. This resulted in two buffered electrolytes: 5.0 m*M* chromate, 20 m*M* Tris, pH=8.5; and 5m*M* chromate, 20 m*M* DEA, pH=9.2. Finally 0.5m*M* TTAB was added to each electrolyte as the EOF modifier. It should be noted that a precipitate forms if the pH of the BGE is below 8. This behaviour is common to chromate BGEs and results from the formation of an insoluble ion pair of hydrogen chromate (pK_{a2} = 6.49) and TTAB.

6.1.3 Results and Discussion

6.1.3.1 Analytical Performance Characteristics of Buffered and Unbuffered BGEs

The analytical characteristics of the buffered and unbuffered chromate electrolytes were compared using six common inorganic anions as analytes: chloride, sulfate, nitrate, chlorate, phosphate, and carbonate. Figure *6.1* shows the electropherograms obtained with the unbuffered pH=8.5 *(a)*, Tris-buffered pH=8.5 *(b)*, and the DEA buffered pH=9.2 *(c)* electrolytes. Comparisons of analytical performances were restricted to the Tris buffered electrolyte. The DEA buffered electropherogram is shown to demonstrate that the buffering of the electrolyte can be achieved with any suitable buffering base that does not introduce a co-anion, and that the pH of the electrolyte can be manipulated by the choice of the base.

Each of the electropherograms in Figure *6.1* shows baseline resolution of all analytes, however the migration times for the solutes are longer for the buffered electrolytes. This can be explained by the lower negative value of EOF mobility for the buffered electrolyte (Table 6.1). Table 6.1 details mobilities of the anions for the Tris buffered and unbuffered electrolytes calculated using the EOF migration time obtained for each sequential run of the anions, so that a drift in migration times for the solutes did not result in a subsequent change in the calculated mobility. The mobilities of each of the anions for the buffered and unbuffered electrolytes show differences that are insignificant.

An important analytical characteristic is the drift of migration times with consecutive injections. Table 6.2 shows data for the change in migration time for successive injections (expressed as a percentage of the absolute migration time for the first injection) for the unbuffered and Tris-buffered electrolytes after 9 successive injections. The unbuffered electrolyte showed a progressive increase in the migration time of each solute, so that by the ninth injection migration times had increased between 1.2-2 %. This drift is likely to be caused by pH changes in the electrolyte reservoirs that occur during electrophoresis as a result of electrolysis

reactions at the electrodes [13, 14]. As expected, no such drift was observed for the buffered electrolyte (changes in migration time of 0.03-0.27%), so the number of injections which can be made without the necessity to replenish the electrolyte was extended significantly.

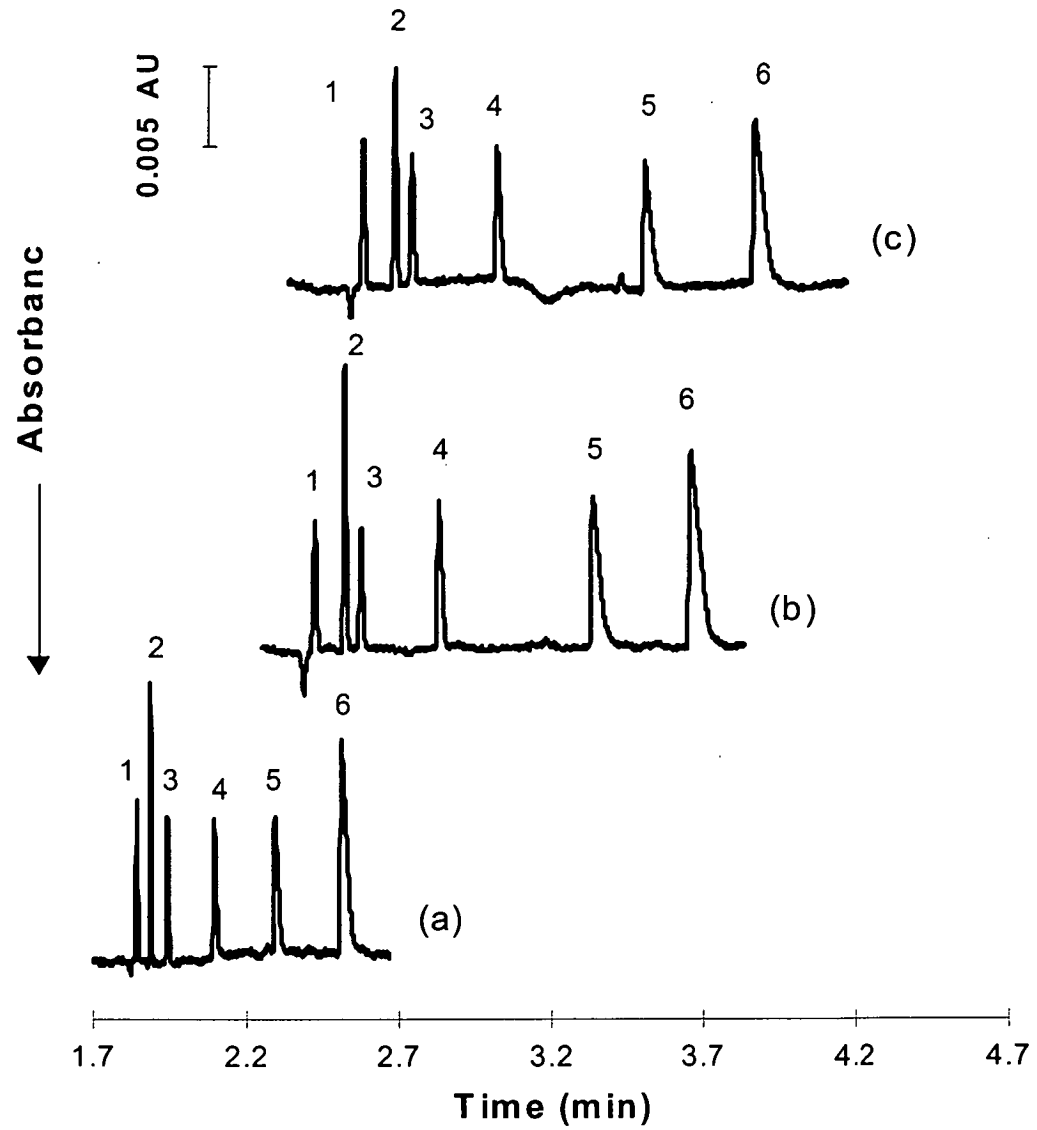


Figure 6.1 *Electropherograms obtained with unbuffered* pH *8.5 (a), Tris-buffered at* pH *8.5 (b) and DEA-buffered at* pH *9.2 (c) chromate electrolytes. Conditions: separation voltage -20kV, indirect detection at 254 nm, hydrostatic injection at 10 cm for 10 sec, temperature 25°C, sample 0.1* mM *of each anion. Key: 1 = chloride,2 = sulfate, 3 = nitrate, 4 = chlorate, 5 = phosphate, 6 = carbonate.*

Table 6.1 **Mobilities of solutes obtained using the buffered and unbuffered chromate electrolytes***

Solute	Unbuffered			Buffered -Tris		
	Mobility (10^{-9} $m^2V^{-1}s^{-1}$)	RSD (%)	Sensitivity (area L mol^{-1})	Mobility (10^{-9} $m^2V^{-1}s^{-1}$)	RSD (%)	Sensitivity (area L mol^{-1})
Chloride	-77.0	0.1	2369	-77.6	0.1	2492
Sulfate	-74.4	0.1	3508	-74.2	0.1	4512
Nitrate	-71.3	0.1	2168	-72.5	0.1	2159
Chlorate	-63.4	0.1	2545	-65.2	0.1	2266
Phosphate	-55.3	0.7	3516	-54.0	0.1	4964
Carbonate	-47.0	0.3	7967	-48.5	0.1	7582
EOF	-34.5	-	-	-8.3	-	-

*Calculations from 9 successive injections. The EOF was determined from the water peak. Conditions: Voltage -20 kV, Injection 10s x 10cm Hydrostatic, Wavelength 254nm, Temperature 25 °C.

Table 6.2 **Effects of buffering of the BGE on repeatability of migration times and peak areas**

Solute	% change in migration time over 9 consecutive runs		Peak area %RSD over 9 consecutive runs	
	Unbuffered	Buffered	Unbuffered	Buffered
Chloride	1.41	0.03	4.5	2.3
Sulfate	1.58	0.03	1.6	1.4
Nitrate	1.69	0.08	4.6	2.6
Chlorate	1.84	0.14	4.8	1.6
Phosphate	1.43	0.24	7.2	3.1
Carbonate	1.97	0.27	7.5	2.8

The repeatability of peak areas is often a problem in CE [18, 19, 20]. Using the same data for the nine injections, Table 6.2 shows a comparison of the peak area repeatability for the buffered and unbuffered electrolytes. The buffered electrolyte gave improved precision of peak areas. Comparison of sensitivity (area divided by concentration) obtained with each electrolyte (Table 6.1) shows no significant differences, which indicates that the addition of the buffer did not affect the performance of the indirect detection. Baseline noise calculated for each electropherogram again showed no significant differences between the two electrolytes.

6.1.3.2 Analysis of Alkaline Samples

An additional advantage of the buffered electrolyte was its tolerance to alkaline sample matrices. The buffering capacity of the Tris electrolyte allowed injection of samples containing up to 50 m*M* sodium hydroxide without loss of separation efficiency, shifts in migration times, or the introduction of interfering peaks (Figure *6.2(a)*). In contrast, the unbuffered electrolyte was intolerant to this level of alkalinity in the sample (Figure *6.2(b)*). The electropherogram for the unbuffered BGE shows a large peak due to the hydroxide ion and distortion of the analyte peaks. An extra peak is also introduced, thereby hampering peak identification.

6.1.4 Conclusions

Buffering of BGEs for indirect detection in CE using a buffering counter-ion of the probe ion has been shown to be an effective approach. Since no competing co-ion to the probe is introduced, the detection sensitivity is not compromised and occurrence of additional system peaks is avoided. The pH of the electrolyte can be manipulated in a wider range by the choice of the buffering base. Buffered BGEs showed very similar separation selectivity for five inorganic anions to that of the commonly used unbuffered BGE, but exhibited superior repeatability in peak areas and migration times and tolerance to alkaline sample matrices. The increased analysis time obtained with the buffered BGEs could be compensated by using a shorter capillary.

This buffering approach may also be applied for electrolytes containing a cationic probe for indirect detection of cations by using a weak acid and titrating it with the cationic probe in the hydroxide form to the *pKa* of the acid.

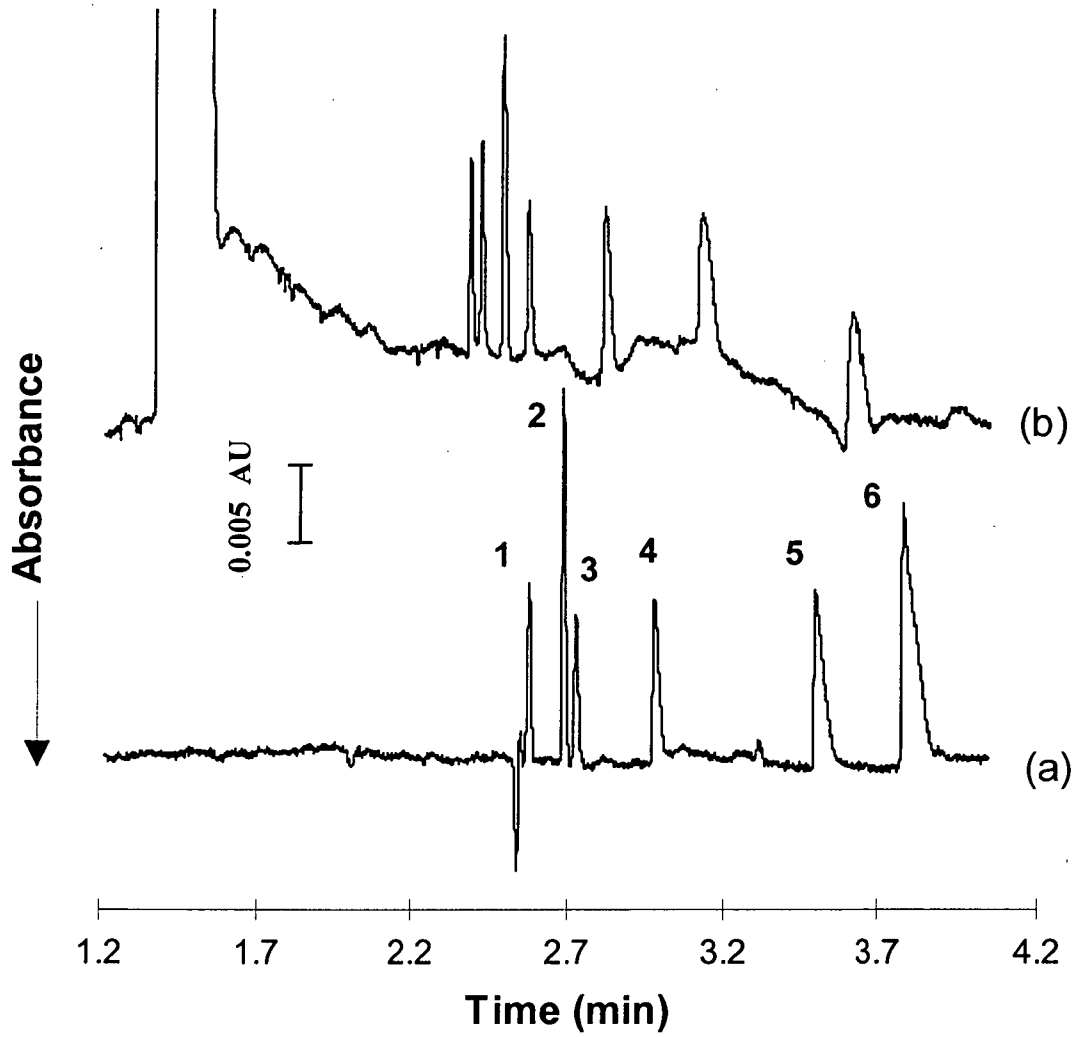


Figure 6.2 *Electropherograms obtained with buffered (a), and unbuffered (b) chromate electrolytes after injection of solutes in an alkaline matrix. Conditions: separation voltage -20kV, indirect detection at 254 nm, hydrostatic injection at 10 cm for 10 sec, temperature 25°C, sample 0.1 mM of each anion in 50 mM sodium hydroxide. Key: 1 = chloride, 2 = sulfate, 3 = nitrate, 4 = chlorate, 5 = phosphate, 6 = carbonate.*

6.2 Co-Anionic Buffered Chromate Electrolytes For Indirect Detection of Fast Anions

6.2.1 Introduction

There are a number of approaches to buffer BGEs for indirect detection of anions. The most common method of buffering is to utilise the probe itself. For this approach the probe is usually a weak acid (for example carboxylic acids such as trimellitic, phthalic, benzoic etc.), and the pH of the electrolyte is kept close to the *pKa* of the probe [21, 22]. The problems with this approach are that: (i) the effective mobility of such a probe will be often low and thus unsuitable for separations of fast analytes, (ii) the optimal buffering capacity is limited to approximately half a pH unit either side of the *pKa* of the probe, so the range of possible BGE pH is narrow, and (iii) the buffering capacity is limited by the concentration of the probe.

Alternatively, electrolytes for indirect detection of anions may be buffered by utilising buffering counter-cations. Typically, these buffers are prepared by titration of the acid form of the probe with a buffering base to a pH close to the *pKa* of the base [13]. The major disadvantage of this method is that the buffering capacity is again limited by the concentration of the probe, which predetermines the concentration of the counter-cation.

Ampholytic buffers were applied in chapter 4 to electrolytes for indirect detection. When a free ampholyte is dissolved in pure water, the pH of the solution is close to the isoelectric point (*pI*) of the ampholyte. An ampholyte will buffer at its *pI* when the *pKa* values of two buffering groups on either side of the *pI* are close together (approx. within one pH unit). The advantage of this approach is that the ampholyte at its *pI* does not decrease the sensitivity by competitive displacement, nor contribute to the conductivity of the solution [23]. However, few ampholytes that buffer well at their *pI* are available, so the choices of BGE pH are again limited.

The problems of limited buffering capacity and limited pH ranges associated with the former buffering approaches seem to be easily overcome by introduction of an

additional buffering BGE co-anion. However, two disturbing effects can occur [16, 24]. Firstly, the presence of an added co-anion induces system peaks, which can interfere with analytes of interest. Secondly, competitive displacement between the probe and the added co-anions can alter the transfer ratio, severely affecting detection sensitivity. The extent to which detection sensitivity is compromised is dependent on the relative mobilities of the probe and co-anion. Although any additional co-anion potentially competes with the probe for displacement by analyte anions, analytes will tend to displace co-anion with the closest mobility [16]. Accordingly, an electrolyte consisting of a fast probe co-anion and a slow competing (buffer) co-anion should be suitable for the analysis of fast analytes, and less appropriate for medium to slow analytes. Indeed, co-anionic buffers such as borate and 2-(cyclohexylamino)ethanesulfonic acid (CHES) have been used successfully for analysis of anions [25, 26, 27, 28, 29, 30]. However, little attention has been paid to the effects of these types of co-anionic buffers on detection sensitivity and to limitations of the analyte mobilities range due to the occurrence of the system peak.

In the following study we investigate the suitability of addition of a relatively slow co-anionic buffer (CHES) to the electrolyte containing a fast probe (sodium chromate) for indirect photometric detection of anions by capillary electrophoresis. Test analytes include fast inorganic analytes (chloride, sulfate, nitrate, chlorate), medium mobility range (phosphate, carbonate), and slow organic anions (propane- and butanesulfonate).

6.2.2 Experimental

The general experimental is given in Chapter Two. Detailed conditions are included in each of the figure captions.

6.2.2.1 Electrolytes

The DEA buffered chromate electrolyte was prepared by titration of a 5 ml aliquot of 100 m*M* chromic trioxide with DEA (*pKa*=9.2) to DEA's *pKa* value. The solution

was made up to 100 ml final volume with Milli-Q water. This resulted in an electrolyte of 5 m*M* chromate, 20 m*M* DEA, pH=9.2.

The CHES buffered electrolytes were prepared by titration of a 5 ml aliquot of 100 m*M* sodium chromate together with a 20 ml aliquot of 100 m*M* CHES with 100 m*M* carbonate free sodium hydroxide solution to the desired pH. The final solution was made up to 100 ml with Milli-Q water. This resulted in electrolytes of 5 m*M* sodium chromate, 20 m*M* CHES, pH=8.5,8.8,9.1,9.4, and 9.7.

Finally 0.5m*M* TTAB was added to each electrolyte as the EOF modifier.

6.2.3 Results and Discussion

6.2.3.1 Choice of Probe and Buffer Co-Ion as a Model Electrolyte

The chromate electrolyte was chosen as the probe in a model system used in this study because it is widely known and used. Furthermore, sodium chromate was an ideal choice as a model electrolyte because it filled the following criteria: (i) chromate is a fast probe, i.e. has high absolute value of mobility, (ii) has favorable detection properties, and (iii) lacks buffering capacity [13].

CHES was chosen as the slow co-ionic buffer for our model system. It is a zwitterionic molecule that consists of a sulfonic acid group and a buffering amino group (*pKa*=9.55). CHES has a net negative charge of -0.1 to -0.9 between the useful buffering pH range of 8.55 to 10.55 (*pKa*±1) (Figure *6.3*). Although the mobility of $CHES^-$ was not available in the literature, most of the analogous Good buffers have mobilities of their fully anionic forms in the range between -22 and -28x10^{-9} $m^2s^{-1}V^{-1}$ [31], and the mobility of CHES is likely to be similar. Within the experimental range of pH 8.5 and 9.7 (Figure *6.3*) CHES has a net negative charge of approximately -0.1 to -0.6, and can be expected to exhibit a relatively slow anionic effective mobility in the range of approx. -2x10^{-9} $m^2s^{-1}V^{-1}$ to approx.-15x10^{-9} $m^2s^{-1}V^{-1}$. With higher pH, the increasingly negatively charged and more mobile CHES has a greater ability to compete with the very fast chromate probe anions for displacement

of the analyte ions and consequently induce system peaks and alter sensitivity. The following series of experiments were designed to investigate the suitability of using CHES as a buffer for the sodium chromate BGE from pH 8.5 to 9.7.

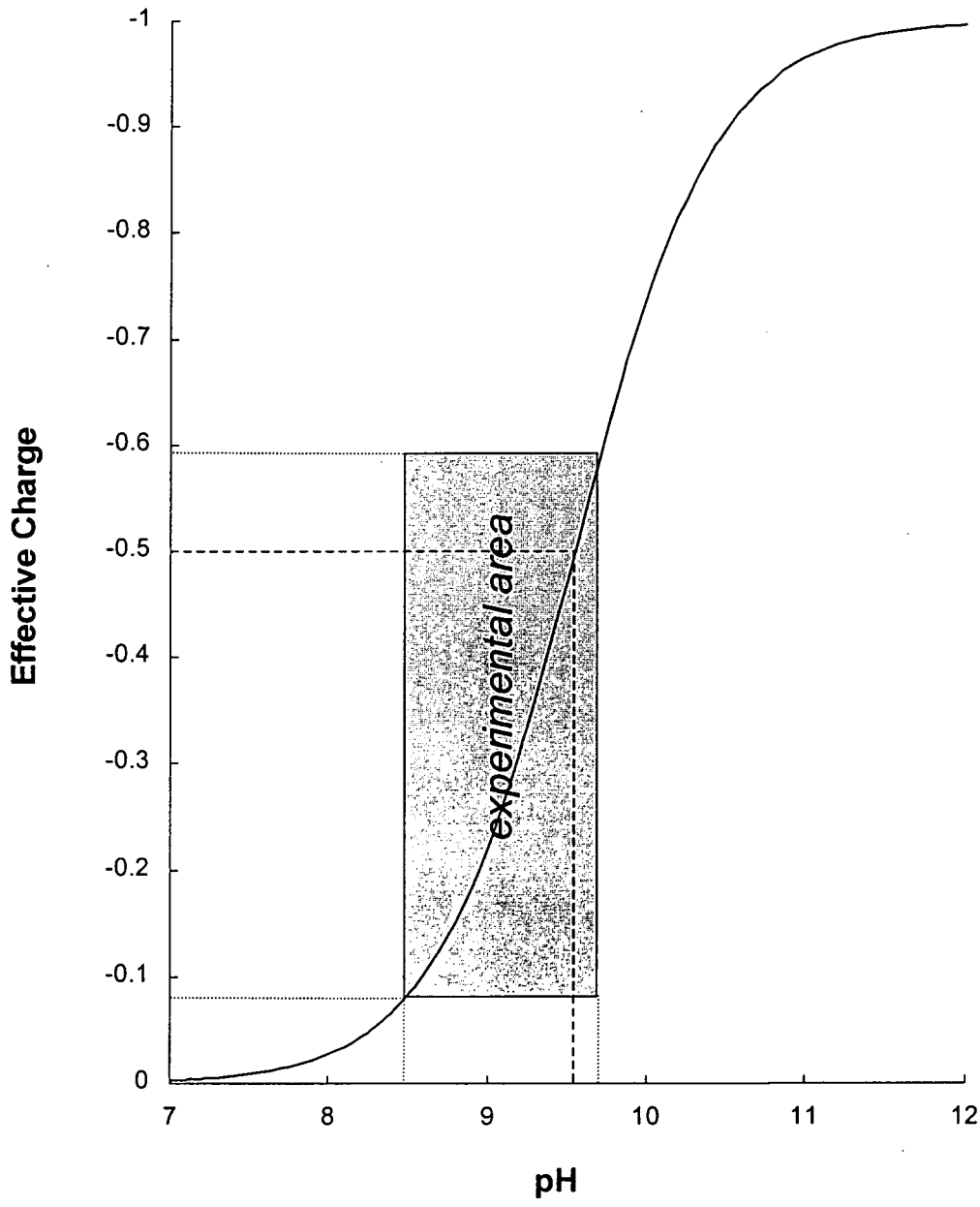


Figure 6.3 *Theoretical plot of effective charge of CHES versus the* pH *of the solution. The grey area denotes the* pH *range and corresponding charge range of CHES in which the experiments were performed.*

6.2.3.2 The effect of the System Peak in the CHES Buffered Chromate BGE

Initially, a control chromate BGE free of major competing co-ions was prepared by titration of chromic trioxide with the buffering base DEA. This electrolyte has been reported to be free of system peaks and provides maximum theoretical sensitivity as the electrolyte consists of a single co-anion . As it is buffered, this electrolyte also displays superior migration time and area reproducibilities than the conventional unbuffered sodium chromate BGE.

Five electrolytes were then prepared containing 5 m*M* chromate and 20 m*M* CHES were prepared at varying pH values (Table 6.3). Figure *6.4* details the electropherograms for eight analytes obtained with each of the BGEs.

The DEA buffered electropherogram shows each of the 8 analytes baseline resolved and free of system peaks. The first CHES BGE at pH 8.5 induced a small system peak that migrated after peak 8. This electrolyte was approximately 1 pH unit below the *pKa* of the buffering amino group. At this pH, the CHES molecule has a charge of -0.08 and mobility of about 8 % of the molecule bearing a full negative charge, but the buffering capacity is only a fraction of the optimum at pH = pK_a (9.55).

At pH 8.8 the induced system peak became larger and migrated closer to peak 8 (butanesulfonate). This BGE was still suitable for the analysis of the analytes, as no interference by the system peak was apparent. However, when the pH was increased to pH 9.1, the system peak completely swamped peak 8 and also distorted the shape of peak 7 (propanesulfonate). At pH 9.4 peak 8 reversed its response and quantitation of peak 7 was impossible. At pH 9.7 peaks 7 and 8 also reversed their responses and the shape of peak 6 became distorted.

In chapter 4 it was shown that the mobility of the system peak is dependent upon the mobility of the co-anions and their relative concentration. As the pH of the electrolyte is moved up towards the optimum buffering pH (*pKa*=9.55), the effective mobility of CHES and its contribution to the ionic strength of the BGE increases. These two effects change the mobility of the system peak from -27.7x10^{-9} $m^2s^{-1}V^{-1}$ at pH 8.5 to -40 x10^{-9} $m^2s^{-1}V^{-1}$ at 9.7 (Table 6.3). This decreases the useful mobility range of the buffer.

Table 6.3 Summary of Analyte and System Peak Mobilities

Analyte	Peak identification	Mobility [a] ($10^{-9}m^2V^{-1}s^{-1}$)	Electrolyte pH [b]	System peak mobility ($10^{-9}m^2V^{-1}s^{-1}$)
chloride	1	-74.6	8.5	-27.7
sulfate	2	-71.8	8.8	-28.3
nitrate	3	-69.4	9.1	-33.9
chlorate	4	-62.0	9.3	-35.4
phosphate	5	-53.1	9.7	-40.0
carbonate	6	-45.4		
propanesulfonate	7	-39.9		
butanesulfonate	8	-31.5		

[a] mobilities of each analyte were measured with 5 m*M* chromate, 20 m*M* DEA, pH=9.2.

[b] each electrolyte consisted of 5 m*M* sodium chromate, 20 m*M* CHES, adjusted to pH with carbonate free sodium hydroxide.

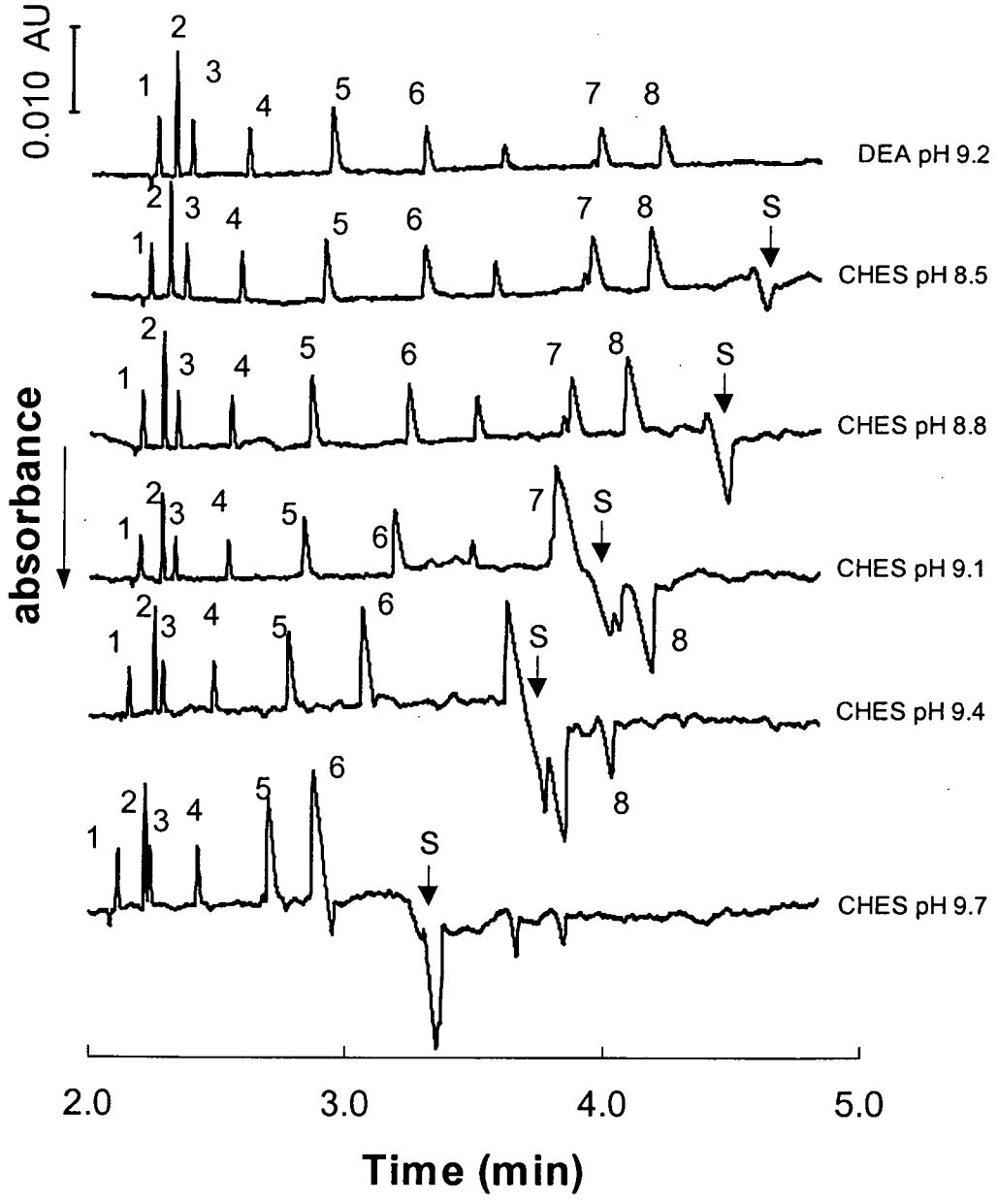


Figure 6.4 *Electropherograms obtained with each of the BGEs. Conditions: electrolytes and peak identities are given in Table 6.3, separation voltage 20 kV, temperature 30°C, detection wavelength 254 nm, 10 s hydrostatic injection at 10cm of standard mixture of 0.1 mM of each anion.*

6.2.3.3 *Changes in Analyte Peak Areas in the CHES Buffered Chromate BGE*

Figure *6.5* details a plot of peak area for all the analytes versus the BGE. The areas for peaks 1 to 4 were all unaffected by the CHES buffer at all pH values. Indeed, there is little difference in the peak areas obtained between the DEA buffered electrolyte and the CHES buffered electrolytes because these analytes were preferentially displacing chromate [16], which has a closer mobility value than CHES. However, the areas of peaks 5 to 8 all increased as the pH of the electrolyte was increased. The increase in area was associated with the peak distortion (broadening) and the extent to which the area increased was dependent upon their proximity to the system peak. These observations were consistent with previous reports on the analysis of anions with BGEs of two or more co-anions [16, 24].

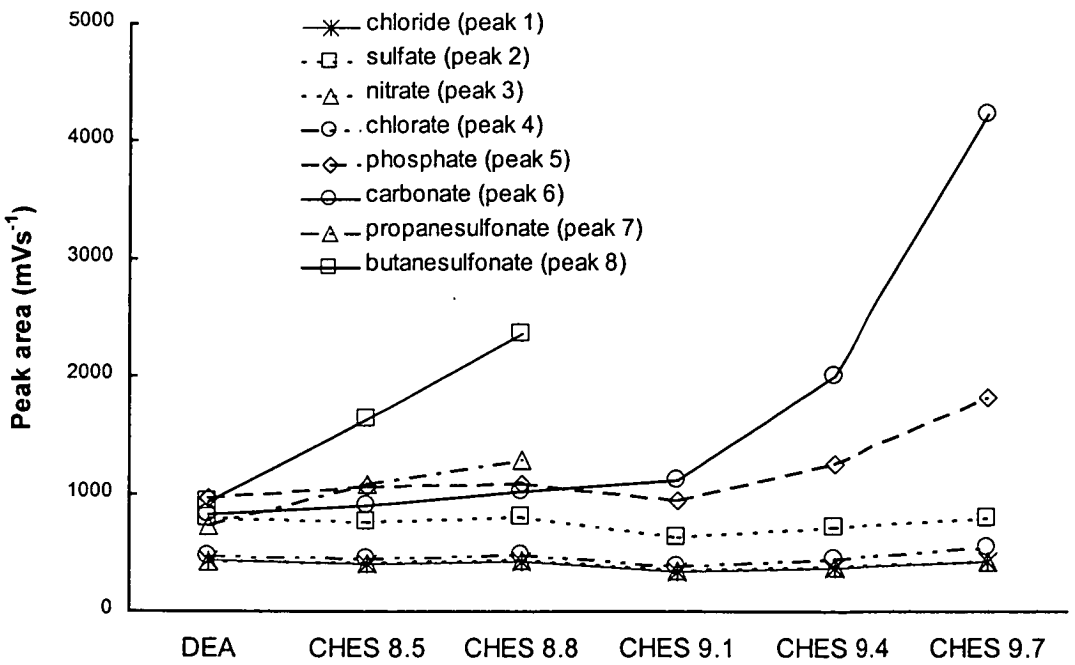


Figure 6.5 *Plot of peak area versus BGE composition. All conditions as in Figure6.4.*

6.2.3.4 Practical Guidelines for the Choice of BGE

The system peak mobility for co-anionic buffered BGEs can be considered the analyte mobility cutoff point. Analytes that migrate at or after the cutoff value are not suitable for analysis. Interference of the system peak can be minimised by maximising the difference in effective mobilities between the probe and the buffer co-anion. This can be achieved by choosing a BGE pH in which the following conditions are fulfilled: (i) the probe is fully ionised and consequently has its highest mobility, and (ii) the buffer co-anion is well below its buffering *pKa*, where its charge and effective mobility are quite low.

However, at these pH values the buffering capacity is a fraction of the optimum. Therefore, the best choice of BGE pH is a compromise where the system peak does not interfere with the slowest analyte of interest, and the BGE has the greatest possible buffering capacity.

It should be noted that when pH = *pKa* of the co-anionic buffer, the BGE has the highest buffering capacity and any increase in pH beyond this value only serves to lessen buffering capacity and increase the effective charge of buffer, which further diminishes the useful mobility range of the electrolyte.

6.2.4 Conclusions

Introduction of slow co-anionic buffers to BGEs with fast anionic probes severely limits the suitability of the BGE for the analysis of slow to medium range mobility anions. System peaks were induced for the model system of sodium chromate buffered with CHES. In the pH range increasing from 8.5 to 9.7, the mobility of the system peak increased -28 to -40x$10^{-9}$$m^2V^{-1}s^{-1}$. The system peak acted as a cut-off mobility for possible analytes. The greatest mobility range of the BGE was at a pH value well below the *pKa* of CHES, limiting the buffering capacity to fraction of the optimum. These trends can be expected to occur for any such indirect BGE in which relatively slow buffering co-anions have been added to the BGE. A compromise between the system peak mobility cutoff for the slow analytes and buffering capacity

of the BGE will always result. A better alternative for buffering of background electrolytes for indirect detection of ions by CE may be in using buffers having an effective charge close to zero, such as buffering ampholytes at their isoelectric point, or buffering with counter cations.

6.2.5 References

[1] W. R. Jones and P. Jandik, *Am. Lab.,* 22 (1990) 51.

[2] C. Francois, P. Morin and M. Dreux, *J. High Resol. Chromatogr.,* 19 (1996) 5.

[3] W. Buchberger and P. R. Haddad, *J. Chromatogr.,* 608 (1992) 59.

[4] W. Buchberger, S. M. Cousins and P. R. Haddad, *TrAC,* 13 (1994) 313.

[5] A. M. Desbene, C. J. Morin, N. L. Mofaddel and R. S. Groult, *J. Chromatogr., A,* 716 (1995) 279.

[6] W. R. Jones and P. Jandik, *J. Chromatogr.,* 546 (1991) 445.

[7] W. R. Jones and P. Jandik, *J. Chromatogr.,* 608 (1992) 385.

[8] P. Jandik and W. R. Jones, *J. Chromatogr.,* 546 (1991) 431.

[9] M. Jimidar, Q. Yang, J. Smeyers Verbeke and D. L. Massart, *TrAC.,* 15 (1996) 91.

[10] S. A. Oehrle, *J. Chromatogr. A,* 671 (1994) 383.

[11] J. Romano, P. Jandik, W. R. Jones and P. E. Jackson, *J. Chromatogr.,* 546 (1991) 411.

[12] R. Kuhn and S. Hoffstetter-Kuhn, Capillary Electrophoresis: Principles and Practice, Springer Laboratory, (1993).

[13] M. Macka, P. Andersson and P. R. Haddad, *Anal. Chem.,* 70, (1998) 743.

[14] M. Macka, P. Andersson and P. R. Haddad, *Poster #116, International Ion Chromatography Symposium, Reading, Sep. 16-19.,* (1997).

[15] J. L. Beckers, *J. Chromatogr. A,* 679 (1994) 153.

[16] T. Wang and R. A. Hartwick, *J. Chromatogr.,* 589 (1992) 307.

[17] G. J. M. Bruin, A. C. Van Asten, X. Xu and H. Poppe, *J. Chromatogr.,* 608 (1992) 97.

[18] A. Vinther and H. Soeberg, *J. Chromatogr.,* 589 (1992) 315.

[19] M. A. Strege and A. Lagu, *J. Liq. Chromatogr.,* 16 (1991) 51.

[20] J. Vinevogel and P. Sandra, *J. High Resolut. Chromatogr.,* 14 (1991) 795.

[21] S. P. D. Lalljie, J. Vindevogel and P. Sandra, *J. Chromatogr. A,* 652 (1993) 563.

[22] F. Y. Guan, H. F. Wu and Y. Luo, *J. Chromatogr., A,* 719 (1996) 421.

[23] S. Hjerten, L. Valtcheva, K. Elenbring and J. L. Liao, *Electrophoresis,* 16 (1995) 584.

[24] J. L. Beckers, *J. Chromatogr. A,* 741 (1996 265).

[25] J. M. Saz, B. Krattiger, A. E. Bruno, J. C. Diez Masa and H. M. Widmer, *J. Chromatogr., A,* 699 (1995) 315.

[26] S. A. Shamsi and N. D. Danielson, *Anal. Chem.,* 67 (1995) 4210.

[27] S. A. Shamsi and N. D. Danielson, *Anal. Chem.,* 67 (1995) 1845.

[28] G. A. Pianetti, M. Taverna, A. Baillet, G. Mahuzier and D. Baylocq Ferrier, *J. Chromatogr.,* 630 (1993) 371.

[29] A. Henshall, M. P. Harrold and J. M. Y. Tso, *J. Chromatogr.,* 608 (1992) 413.

[30] R. Neubert, K. Raith and J. Schiewe, *Pharmazie,* 52 (1997) 212.

[31] F. Foret, L. Krivankova and P. Bocek, Capillary Zone Electrophoresis, VCH Veragsgesellschaft mbH; Weinheim, (1993).

7. General Conclusions

The following general conclusions can be made regarding the separation and indirect detection of anions by capillary electrophoresis.

The sensitivity of indirect detection could be optimised for any given probe by consideration of factors that affected the value of the transfer ratio. Of primary importance, BGEs that contained U.V. transparent co-anions were undesirable due to potential competitive displacement of the probe, which decreased the transfer ratio and reduced the sensitivity. Potentially competitive anions were avoided by: (i) choice of correct buffer, such as a buffering base, and (ii) addition of EOF modifiers in the hydroxide form.

Of secondary importance was the choice of counter cation. A low mobility counter cation was desirable to minimise the decrease in transfer ratios for anions that migrated faster than the probe when compared to a high mobility counter cation such as sodium. Fortunately, buffering bases were of low mobility, serving the dual function of providing buffering capacity to the BGE, as well as maximising the transfer ratio for a given set of analytes.

For BGEs that consisted of more than one co-anion, an analyte displaced preferentially the co-anion component of the BGE for which its mobility was closest, and displaced exclusively components that had the same mobility. This behaviour allowed peak shapes to be controlled by choosing multiple probe anions that matched the mobilities of the analytes. A practical problem with multiple component electrolytes was the inducement of system peaks, which had the potential to interfere with analytes of interest. The mobility of the system peaks was manipulated by selection of the relative concentrations of each probe, providing a simple method to optimise the BGE for analysis of any given analytes. Multiple probe electrolytes may be potentially applied to samples that contain analytes that have a wide mobility range.

Highly absorbing dyes were successfully utilised as probes to decrease the limit of detection by at least an order of magnitude compared to conventional probes. Buffering these types of electrolytes was achieved with ampholytic buffers or with

buffering bases. Buffers that introduced co-anions were unsuitable because the detection sensitivity was compromised and potentially interfering system peaks were induced.

The detection sensitivity was further increased by a factor of three when a Z cell was utilised as the detection cell. It was necessary to use low concentrations of probe to keep the background absorbance within the linear range of the detector, rendering buffering with a counter cation base unsuitable. The only viable means to buffer the BGE was to employ an ampholyte.

Superior reproducibilities of peak areas and migration times resulted when BGEs were buffered. Further, the buffered electrolytes had a higher tolerance to alkaline sample matrices. Two buffering approaches were demonstrated for the analysis of inorganic anions. The first and best approach was to utilise a buffering base. Since no competing co-ion to the probe was introduced, the detection sensitivity was not compromised and occurrence of additional system peaks was avoided. The pH of the electrolyte could be manipulated in a wider range by the choice of the buffering base.

The second approach introduced slow co-anionic buffers to BGEs with fast anionic probes. System peaks were induced in the model system, severely limiting the suitability of the BGE for the analysis of anions of low to medium mobility. Further, for minimal interference of the high mobility anions, the pH of the BGE was kept well below the *pKa* of the buffering agent, providing a fraction of the possible buffering capacity.

Finally, it should be noted that further research is required in the following areas. Mathematical modelling of the mobility of the system peak for multiple component electrolytes would be useful in aiding the design of BGEs for the analysis of analytes of a given range of mobilities.

BGEs that contain highly absorbing dyes as probes have the probe present in very low concentrations, making buffering of the BGE difficult unless an ampholyte is used. At present, few ampholytes exists that buffer well at their *pI* so the choice of BGE pH is limited. Therefore, it can be expected that further research would be

directed towards synthesising new ampholytic buffers with desired *pI* and pH buffering values. Further, this project has investigated only a limited number of dyes as probes, so there is potential to investigate other commercially available dyes for characteristics such as better mobility matching for fast anions to improve peak shapes, and higher molar absorptivities to improve detection sensitivity. Cationic dyes may be also applied as probes for the analysis of cations.

Although the results from this thesis suggest that co-anionic buffers are not optimal for the analysis of anions, application of near zero mobility co-anionic buffers may be useful for the analysis of fast anions with fast probes. It is envisaged that these types of buffers may be formed using high molecular weight organic acids.